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Journal of
ECONOMIC ENTOMOLOGY

Published by the
ENTOMOLOGICAL SOCIETY OF AMERICA

VOLUME 53 December : 1960 NUMBER 6

JOURNAL OF ECONOMIC ENTOMOLOGY

Published by

ENTOMOLOGICAL SOCIETY OF AMERICA

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A bimonthly journal published on the 15th of February, April, June, August, October, and December, devoted to the interests of economic entomology. The authors are entirely responsible for statements in the *Journal*, whether of fact or opinion.

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Second-class postage paid at Menasha, Wisconsin. By order of December 19, 1958.

PRINTED BY GEORGE BANTA CO., INC.

MENASHA, WISCONSIN, U.S.A.

Issued Dec. 27, 1960

JOURNAL OF ECONOMIC ENTOMOLOGY
ENTOMOLOGICAL SOCIETY OF AMERICA

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JOURNAL OF ECONOMIC ENTOMOLOGY

ENTOMOLOGICAL SOCIETY OF AMERICA

Vol. 53 December, 1960 No. 6

Chemical Control of the Eastern Spruce Gall Aphid with Observations on Host Preference and Population Increase[1]

JAMES W. BUTCHER and DEAN L. HAYNES, *Michigan State University, East Lansing*

ABSTRACT

Field insecticide studies demonstrated that lindane (1 lb. actual toxicant per 100 gallons of water) gave good control of eastern spruce gall aphid (*Chermes abietis* L.) when applied between the time of last freeze and start of terminal shoot elongation. Sevin® (1-naphthyl methylcarbamate) (2 lbs.) was less effective, while dimethoate (2 lbs.), malathion (1 lb.), and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) (1 lb.) gave little or no control. Statistical analyses of infestations over a 2-year period show (1) a 15% greater incidence of attack on white as compared with Norway spruce; (2) a 6% increase in over-all infestation from 1958 to 1959; (3) a strong tendency for infestations to recur on previously infested trees. This suggests that by harvesting infested trees, a grower could reduce the infestation in a given planting, if such a program were initiated before the percentage of attacked trees was too high. Finally, the view that certain trees are resistant to aphid attack is questioned, using as a basis the tendency of this insect to reinfest previously infested trees. It is reasoned that, even though new and previously uninfested trees appear to be randomly attacked each year, the rate of spread is so slow that one-fifth of the trees in the study area are expected to remain uninfested for 5 consecutive years, owing to insect behavior alone.

The eastern spruce gall aphid, *Chermes abietis* L., produces pineapple-shaped galls at the bases of new shoots of white spruce, *Picea glauca* (Moench) Vass., and Norway spruce, *Picea excelsa* Link, in Michigan. Christmas trees which have numerous galls at the time of cutting must be sold at a reduced price or withheld from sale until new growth has concealed the injury.

According to Wilford (1937), the eastern spruce gall aphid infests Norway and white spruce trees, but has no secondary host in this country. He also states that the insect has a 1-year asexual life cycle and that the various life stages overlap.

CHEMICAL CONTROL EXPERIMENTS.[2]—In efforts to evaluate promising insecticides for control of this insect, interplanted rows of Norway and white spruce, 4 to 6 feet high, growing near Lake City, Michigan, were selected for experimentation. In order to ascertain the importance of timing, three spray dates were selected—April 21, April 30, and May 14. Six treatments (table 1) were applied to 15-tree plots, randomly selected within each of the four replications for each date. A single untreated row separated the blocks of treatments for each date. All insecticides were applied with a 2-gallon Lofgren sprayer equipped with a fan-type nozzle and operated at 15 to 25 lbs. pressure. Trees were sprayed until they were visibly wet, but spraying was discontinued before run-off occurred.

By April 30, most of the aphids were covered by a white, woolly material. By May 14, eggs were present and some white spruce bud scales had split or were pushed off.

In September, the galls present in the top 30 inches of the tree crown were counted. Two observers, one on each side of the tree, made counts simultaneously, and the total for each tree was recorded. If no galls were found on the top 30 inches, the entire crown was examined and presence or absence of galls recorded for the purpose of determining the number of trees of each species infested.

Resistance of certain trees to injury by this pest is generally accepted. Wilford (1937) observed that 39% of the trees on the Saginaw Forest escaped attack over a 10-year period of infestation even though these trees had their branches intertwined with infested trees. The 15-tree plot size used in these studies was considered large enough to minimize the influence of resistant trees on the interpretation of results. Even so, when data for each of the three dates were subjected to independent analysis of variance, the variation between replications was too great to permit valid comparisons of applications made on April 30 and May 14.

The entire experiment was subjected to a multiple analysis of variance so that a better estimate of error could be obtained. The results showed that there were no significant differences (in insecticide performance) that could be attributed to time of application, and that the time of application did not significantly interact with treatment to produce differences in gall abundance. As a result, the four replications for each spray date were

[1] Journal Paper No. 2619. Accepted for publication May 2, 1960.

[2] The writers gratefully acknowledge cooperation given by the Departments of Forestry and Zoology, Michigan State University and Albert Engel, Engelwood Tree Farms, Lake City, Michigan. The following firms gave welcome assistance upon request: American Cyanamid Company, Shell Chemical Corporation, Union Carbide Chemical Company and the California Spray Chemical Corporation.

Table 1.—Effect of applications of insecticides against the eastern spruce gall aphid.

Insecticide	Formulation (lb./gal in E.C.)	Pounds Toxicant per 100 Gal.	Mean No. Galls[a] per Tree
Dimethoate	4	2	23.8
Malathion	5	1	17.3
Phosdrin	2	1	15.2
Untreated			13.6
Sevin, 50%	W.P.	2	7.4
Lindane	1	1	2.7

[a] Time of application disregarded; 12 replications per treatment. Treatments with vertical line in common do not differ from each other at the 5% level of significance (Duncan 1955).

lumped together into 12 replicates. Using the error term computed for the entire experiment, the means were sequentially compared using the new multiple range test described by Duncan (1955) (table 1). Of all the materials used, only lindane differed significantly from the check at the 5% level. This is not unexpected in view of the work reported by Fowler & Gair (1956) who found that 0.05% BHC sprays gave good control, whereas results with 0.05% nicotine, 0.05% endrin and 0.01% parathion sprays were characterized as less effective or disappointing. In figure 1, performance of the insecticides is shown as per cent increase or decrease in numbers of galls, using the untreated check plot counts as a base. In this graphic presentation of the data, a consistent pattern emerges.

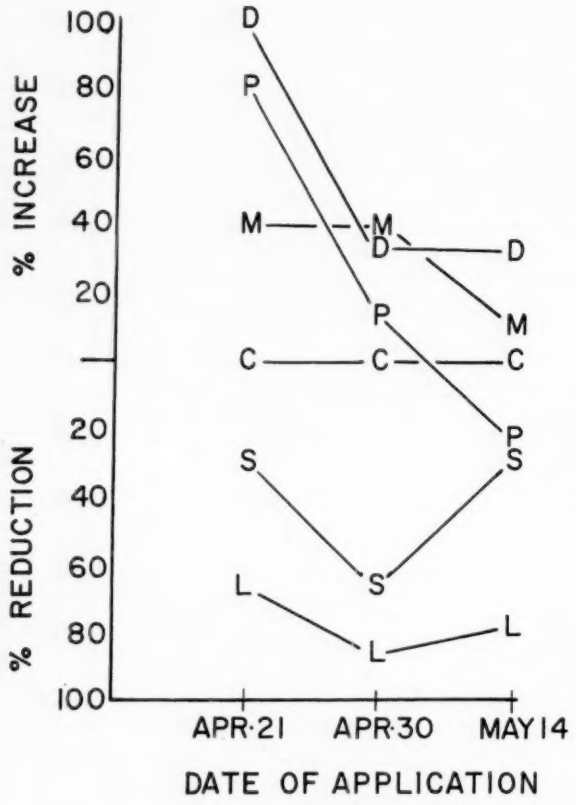

FIG. 1. Per cent reduction or increase of eastern spruce gall aphid populations in relation to date of insecticide application. (D = dimethoate; P = Phosdrin; M = malathion; S = Sevin; L = lindane; C = check)

The fairly uniform results obtained with lindane and Sevin® (1-naphthyl methylcarbamate) at all three times suggests that a grower has some latitude in selecting a spray date as long as it falls between the time of last freeze and gall formation in the spring. The fact that higher populations were found on the dimethoate, malathion, and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) than were present in the untreated checks might be attributed in some degree to experimental error. The maximum temperatures recorded for each of the spray dates were 53° F. (April 21); 55° F. (April 30) and 47° F. (May 14). Corresponding minimum readings were 22° F., 33° F. and 37° F., respectively. It is possible that the low temperatures prevailing at the time of application adversely affected performance of the phosphate compounds. Such a consistent pattern, however, invites consideration of the possible deleterious effects these materials may have on natural enemies of the eastern spruce gall aphid.

HOST PREFERENCE AND HOST REINFESTATION RATES.—After the samples were collected, a relatively large population gradient was apparent from one end of the plantation to the other. This gradient contributed considerably to the experimental error in the insecticide trials, because it was contained within each replication and could not be observed and corrected for from one replication to the other. However, this source of error was greatly minimized by the use of relative proportions of affected trees within each plot.

Since a large number of trees were examined (1079), it was possible to evaluate four biological considerations: (1) aphid feeding preference between Norway and white spruce, (2) the rate of population increase, (3) the tendency to reinfest previously infested trees and (4) the tendency to attack new, previously uninfested hosts.

1. In this study, there were 437 white and 642 Norway spruce distributed throughout the plots in such a manner that neither species was absent from any plot. All trees were scored as either "infested" or "uninfested."

Before the analysis of these data could be made, the influences of the population gradient and insecticide applications had to be established. By applying a chi square test for heterogeneity, it was shown that the population gradient and insecticide treatment did not make the relative proportion of attacked white and Norway spruce deviate significantly from plot to plot. One would expect to find heterogeneity between plots as great as or greater than that observed, between 10 and 25 times in a hundred, even if there were no systematic differences in the relative proportions of infested white and Norway spruce from plot to plot. Therefore, the hypothesis that the various plots are homogeneous with regard to the relative proportions of infested white and Norway spruce cannot be rejected at the 5% level of significance. The lack of significant heterogeneity suggests that the total number of infested and noninfested Norway and white spruce could be analyzed using a chi square test with one degree of freedom. The results showed a highly significant preference for white spruce.

Since a chi square test for significant difference can be influenced strongly by a single unknown variable, a more

sensitive test completely independent of chi square was run. This consisted of using the difference between the proportions of affected white and Norway spruce attacked within each plot.[2] The difference between these proportions was analyzed, using paired observations within each plot. The "t" distribution was used to test if the mean of the difference was significantly different from zero. The value of "t" obtained was 2.85, which with 17 degrees of freedom was significant at the 5% level, indicating that there is a real difference between the proportion of infested white and Norway spruce. The two-tailed 95% confidence limits for the difference between the proportions of white and Norway spruce is greater than 9% and less than 20%. Thus, in a mixed stand of Norway and white spruce one would expect to find approximately 15% more white spruce attacked than Norway. Whether this exact situation would hold up under condition of pure stands of Norway or white spruce cannot be stated. Though the risk probability cannot be stated definitely for pure stands, it undoubtedly is greater for white spruce than for Norway spruce.

2. As previously stated, there is general agreement that the host exhibits differential resistance to this pest and that many trees will remain uninfested throughout the course of an outbreak. These inferences are seldom drawn from quantitative data but are usually made from general impressions of stand appearance.

In an effort to measure this tendency each tree in the plot was scored as to whether it was attacked in 1958, as indicated by old galls, and whether the same tree was reattacked in 1959.

Table 2 presents the relative proportions of the various categories of infestation for 1958 and 1959. This shows that the population increase was 6% of the total trees; however, it was a 15% increase if just infested trees are considered. In order to determine if this was a real increase in population it was necessary to determine if the number of infested trees which became uninfested was significantly less than the number of uninfested trees which subsequently became infested. A chi square test of this showed that the increase in population was far greater than could be accounted for by sampling error.

3. The original data for table 2 were subjected to a contingency chi-square analysis to determine if the 1958 infestation significantly influenced those trees attacked in 1959. An extremely large chi-square was observed (chi-square-$262/n=1$) in this contingency test. This means that the trees infested in 1958 had a great influence on those trees infested in 1959. This statement is also true for the uninfested trees. The influence is most easily observed when it is pointed out that 74% of the 1958 infested trees were reinfested in 1959 and that this reinfestation constituted 64% of the total 1959 infestation. The expanding population (in terms of attacked trees) caused the reinfested trees to show up as a lower total percentage of the 1959 population. If the aphids were only maintaining their number of attacked trees in the stand, the two percentages would be equal.

Table 2.—Proportion of infested and uninfested spruce in the study area during 1958 and 1959.[a]

	1959		Total (%)
Per Cent	Infested (%)	Uninfested (%)	1958
Uninfested 1958	15	49	64
Infested 1958	27	9	36
Total 1959	42	58	

[a] Proportions expressed as a per cent of 1079 trees observed.

Table 3.—The estimated effect of harvesting in the study area over a 3-year period, based on observed population data for both 1958 and 1959.

		Estimated Number of	
Date	No. of Trees Removed	Infested Trees Remaining for Following Year	Trees Removed Which Would Not Have Been Reinfested the Following Year
1958	394	164	101
1959	164	59	43
1960	59	21	15
Total	617		159

This strong tendency to reinfest previously attacked trees suggests a method for control of this insect in Christmas tree plantations. Normally, Christmas trees are harvested in spruce plantations over a period of 2 to 4 years. At first, small "table trees" are harvested and other trees are removed as they reach a prime degree of development and market conditions are best for the grower. Galls on a tree lower its grade and growers may postpone cutting the tree so that next year's growth will obscure the presence of galls. However, if infested trees were harvested (even at a loss), this approach conceivably could virtually eliminate the pest over a period of 3 years, even under conditions where it was expanding to new trees. Table 3 shows what the influence on the aphid population would have been, assuming the same population increase for 1960 that was observed between 1958 and 1959. The actual rate of increase is undoubtedly overestimated since the population remaining after removing galled trees the first year is considerably less than it was when the increase in population was estimated. The loss due to degrading galled trees would also be considerably less since the appearance of only a few galls on a spruce would not affect its acceptance in the market place.

4. Insofar as the tendency to attack new, previously uninfested hosts is concerned, the data suggest an alternative to genetic tree resistance as an explanation for apparent susceptibility of certain trees to attack. The observed tendency for reinfestation of attacked trees was .64. If this proportion remained constant it can be shown that 11% ($.64^5$) of the infested trees would be attacked for 5 consecutive years. If these same proportions were extrapolated to a population where 50% of the trees in the plantation were attacked each year, 19% of the trees would have escaped attack during the same 5-year period. An assumption must be made here, however, that each year's newly attacked trees are infested at random. It was possible to obtain some information from the sample which supports this assumption. Since this insect appears to be somewhat sedentary in its habits, one might expect that

[2] In order to insure a normal distribution, the relative proportions of affected spruce were subjected to an arcsin transformation as described by Snedecor (1957).

those trees adjacent to infested trees would more likely be infested than those in another part of the plantation. This could result in a contagious rather than a random distribution of tree to tree infestation. A one-sample runs test (Siegel 1956) was applied to the sequence of infested and noninfested trees as they appeared in the rows. There was no information available for relationship across rows. The results showed that the 559 observed runs were not significantly different from the mean expected number of runs of 542. The two-tailed probability for rejecting the hypothesis of randomness did not approach significance at the 5% level. It appears that the proportion of newly attacked trees in this plantation are infested at random, and this is reflected in the over-all distribution of infested trees.

The data presented here suggest that much of the apparent immunity reported by early workers can be accounted for by insect behaviour, which at best, is poorly understood.

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Insectary Production of *Stethorus* Species[1]

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ABSTRACT

A program of mass production and periodic release of imported *Stethorus* sp., a predator of tetranychid mites, is being conducted by the University of California Department of Biological Control. Large numbers of the Pacific spider mite (*Tetranychus pacificus* McG.) are reared on linted oranges, under controlled conditions. The mite-infested fruit is used to mass-produce the *Stethorus* sp. in specially designed rearing units. The *Stethorus* adults are collected from the units and released on mite-infested plants.

Members of the genus *Stethorus* are widely distributed predators of tetranychid mites and are often effective in controlling outbreaks of these mites. Other predators, such as predaceous mites, thrips, and green lacewings, are instrumental in maintaining mite populations at low densities, but generally they are not effective in controlling high-density populations of mites.

Although native species of *Stethorus* are usually present in areas infested with tetranychid mites, it may be desirable to introduce new *Stethorus* species into an environment. Such characteristics as cannibalism, searching capacity, weather tolerance, mite species preferred, and mite-density requirements might favor one species in one microhabitat and another species in another microhabitat. Therefore, introduction of a new *Stethorus* may improve or extend an existing biological balance.

The mass production of *Stethorus* species requires a tremendous supply of host mites. Fleschner (1950) found that *Stethorus picipes* Casey required at least 135 mites to complete development, and that the larvae were capable of consuming up to 486 mites each. If we estimate that 300 mites are required for oviposition and larvae development, then approximately 6,000,000 mites would have to be produced to rear 20,000 *Stethorus* adults, which is the present monthly production at the University of California's Riverside insectary.

Several species of mites have been used for rearing *Stethorus* species. Finney (1953) used the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), and Fleschner (unpublished data) used the spider mite, *Tetranychus cinnabarinus* Bois. The Pacific spider mite, *Tetranychus pacificus* McG., has proved to be superior for mass-production purposes at the Riverside insectary. It produces tremendous populations on Valencia or navel oranges, and it is not sensitive to trace pesticide residues on the fruit. Purifying the air with activated charcoal is not necessary with this mite, as is the case with the insectary rearing of certain other species of tetranychid mites.

METHODS AND MATERIALS.—Washed, waxed, and graded oranges are obtained from a packing house and placed in cold storage (45° F.) until needed. Fruit which has been treated with ethylene gas to improve its color is not satisfactory because it frequently decays too soon under insectary conditions. The fruit is prepared for mite infestation by first being placed on $15\frac{3}{8}\times28$-inch hardware-cloth trays which hold 55 size 138 fruit (fig. 1). Briefly, the cold fruit is held in a steam cabinet for "sweating" (fig. 2). Then, the tray of moist fruit is placed in a linting box and flocking lint is applied to the fruit (fig. 3). About 25 ml. of flocking is distributed over the curved bottom of the linting box, a horizontal perforated tube 1 inch above the curved bottom is connected to air pressure, and the flocking is blown up through the hardware-cloth tray and onto the fruit.

Two colors of flocking lint are used to distinguish the DDT-treated from the untreated fruit. All trays of fruit used to sustain the mite culture are linted with a mixture of 10% DDT (standard 50% DDT WP is used), 10% diluent, and 80% green flocking by weight. This prevents various mite predators, particularly predaceous mites,

[1] Paper No. 1211, University of California Citrus Experiment Station, Riverside, California. Accepted for publication May 2, 1960.

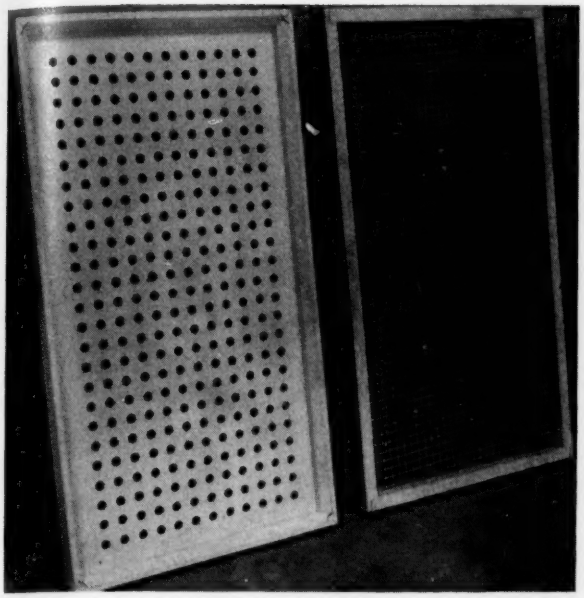

FIG. 1.—Left: Perforated masonite tray for rearing of *Stethorus* on mite-infested oranges.

Right: Hardware-cloth tray for linting and infesting oranges.

from contaminating the mite culture. Since *Stethorus* adults are sensitive to DDT, the trays of fruit used in the *Stethorus* oviposition units are linted with white DDT-free flocking. The flocking lint is composed of viscose rayon fibers "Verlon F21" and it provides a light, fuzzy covering over the fruit which encourages the female mites to commence feeding and ovipositing.

Routine for Operating Mite Culture.—To provide the *Stethorus* culture with a predictable and constant supply of mite-infested fruit, it was necessary to establish a standardized routine for operating the mite culture. The culture is kept in a dark room where the temperature is

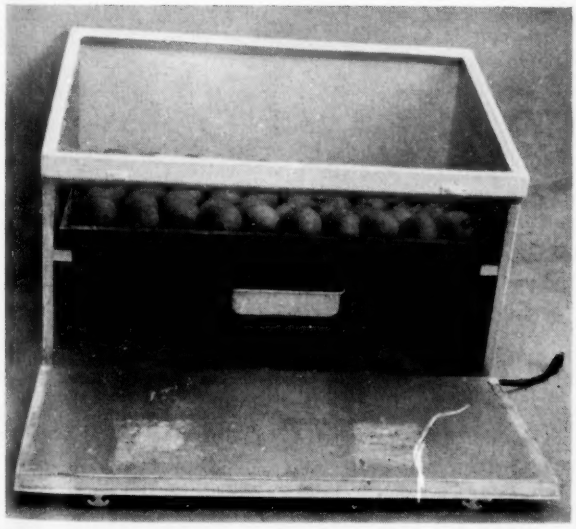

FIG. 2.—Steam cabinet containing hot plate, water pan, and a tray of fruit.

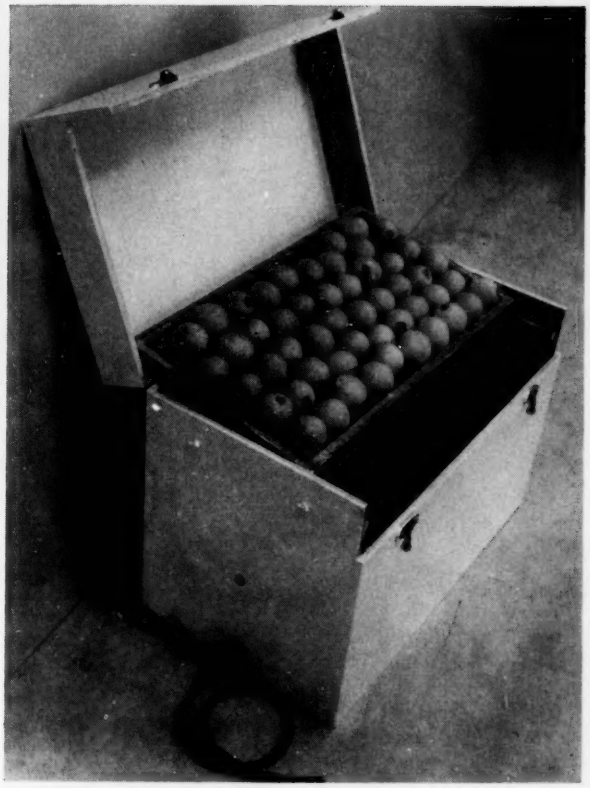

FIG. 3.—Linting box, showing curved bottom, perforated air tube, and a tray of fruit ready for linting.

maintained at 78° to 80° F. and the relative humidity at 45% to 55%. All of the mite culture operations are done on Monday, Wednesday, and Friday. The culture is divided into four groups, each consisting of one or more units. Each unit is a tray of fruit heavily infested with mites which usually has on top of it a fresh tray of fruit ready to be infested (fig. 4). The operations conducted on each group are staggered so that no two groups have the same operations on the same day. A typical operation sequence for one group would be to place trays of DDT-linted fruit for infesting on the units Monday, remove the trays of spent fruit Friday, and add trays of untreated fruit the following Monday for infesting.

Each unit remains as an individual mite culture. This reduces the chance of contamination and also allows for flexibility. The following procedure represents the handling of one unit. First, a tray of DDT-treated fruit is placed on top of the tray of heavily mite-infested fruit; the mites move upward by negative geotropism onto the new fruit. Four or five days later the tray of old fruit is removed and replaced by the newly infested tray of fruit. The new culture of mites is allowed to incubate for 2 or 3 days. Then, a tray of DDT-free linted fruit is placed on top of the incubating culture. The DDT-free fruit is infested for 4 or 5 days; then it is replaced by a tray of DDT fruit, which completes one cycle. One cycle can take 11 to 14 days, depending upon the position of weekends in the cycle. The life cycle for the mite at this temperature is about 10 days. A standard flashlight is useful to determine

FIG. 4.—A rack containing six mite units divided into two groups: one group is incubating, the other is infesting.

FIG. 5.—*Stethorus* rearing unit. The upper cage contains developing predator larvae, and the closed lower cage contains an oviposition unit.

the progress of the mite cultures. When the light is held at the proper angle, the mites and their webbing show clearly.

The Rearing Unit.—The *Stethorus* rearing unit (fig. 5) is a multipurpose cabinet. The top part with sliding doors is for equipment storage, the two large cages are for rearing, and the bottom part is for tray storage. The cabinet is on casters, which allow rapid movement from room to room or to the fumigator. The cabinet is 74 inches high, 34 inches wide, and 19 inches deep. The two rearing cages are each 31 inches wide and 23 inches high. The back of the cages is covered with organdy cloth and the front is closed with a 32×24-inch plywood door.

The trays used to hold fruit in the rearing units are the same size as the trays used for infesting the fruit with mites. However, the tray bottom is made of perforated masonite (fig. 1) instead of hardware cloth. The masonite gives better support to the fruit in the emergence units. These trays are also easier and cheaper to make and last longer than the hardware-cloth trays.

Operation of Stethorus Culture.—The operation of the *Stethorus* culture is also standardized; however, considerably more flexibility must be allowed to utilize the fruit to maximum advantage. All *Stethorus* rearing is done at about 83° F. which is usually the highest temperature that the fruit can withstand without excessive drying and breakdown. The trays of infested DDT-free fruit are allowed to incubate for several days after removal from the mite culture, then the fruit is used in *Stethorus* oviposition units. A *Stethorus* oviposition unit (fig. 5) uses one tray of well-infested fruit with an abundance of mite eggs and usually 500 well-fed adult *Stethorus*. The *Stethorus* feed on the mites and oviposit for 4 days. Then the *Stethorus* adults are removed with a vacuum aspirator. After the *Stethorus* eggs hatch, additional trays of mite-infested fruit are added to the original tray when the *Stethorus* larvae begin to search actively for mites (fig. 5). The feeding of the young larvae is extremely critical; the tiny larvae must have an abundant supply of mite eggs, larvae, and nymphs. The first-instar larvae are very weak and slow and usually they cannot overpower an adult mite. After the larvae reach the third instar, they can move rapidly from fruit to fruit seeking mites. Old fruit, previously treated with DDT, may, in some cases, be used at this time. Some species of *Stethorus* larvae have considerable resistance to DDT, and the emerging adults are not seriously affected by the old DDT residue. Mites should be readily available to the larvae prior to pupation or considerable mortality will result in the first few hours after emergence.

The adult female *Stethorus* can lay about 6 eggs per day and since the sex ratio is about 1:1, an oviposition unit using 500 *Stethorus* for 4 days theoretically could produce 6,000 eggs, a 12-fold increase. Many adverse factors operate to reduce this ratio. Cannibalism is a constant problem which can be partially reduced by supplying an

overabundance of mites. Other factors, such as mechanical injury during fruit handling, starvation, and emergence mortality, contribute to lowering efficiency. A 7-fold increase is the highest obtained so far.

When emergence begins, a light is located behind the cloth back of the emergence unit. The young adult *Stethorus* fly to the illuminated back of the emergence unit where streaks of honey on an enameled aluminum strip are provided as a supplementary food (fig. 5). A vacuum aspirator is used to collect the *Stethorus* adults into large pyrex test tubes. When the *Stethorus* are to be released in the field, they are collected 500 to a test tube, and a strip of enameled aluminum streaked with honey is slipped into each tube. The open end of the tube is covered with fine-mesh nylon cloth for ventilation. The tubes of *Stethorus* are taken to the field in a small evaporative cooler and released on mite-infested plants.

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Some New Mutants and Linkage Groups of the House Fly[1]

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ABSTRACT

A search for new mutants in the house fly, *Musca domestica* L., has been continued and a list of seven new marker strains is included in this paper. They are four eye color mutants, *car* (*carnation*), *cm* (*carmine*), *rb* (*ruby*) and w^{au} (*auburn*); an eye shape mutant, *ro* (*rough*); a wing shape mutant, *ct* (*cut*); and a wing vein mutant, *Lp* (*Loop*).

Linkage tests to classify 17 marker genes found in this laboratory and obtained from other investigators have been performed. Five linkage groups, which are assumed to represent all the autosomes of the house fly, have been demonstrated. No sex-linked mutant has yet been detected.

The discovery of house flies, *Musca domestica* L., resistant to DDT in the late 1940's has led to numerous investigations of insecticide resistance in this and other insects. Genetic investigation of the organism involved is an important and fundamental step toward an understanding of the problem. A number of genetic studies of the resistance problem in the house fly have been reported; however, conclusive results are very few, in part owing to a lack of basic genetic knowledge of this insect. Therefore, a study of the genetics of the house fly with an emphasis on the accumulation of marked chromosomes seemed of great importance.

Since 1953 some useful mutants of the house fly have been reported by Milani and other investigators. More mutants are necessary, however, to further our knowledge of the genetics of this insect and to make possible more refined studies on the inheritance of resistance. We initiated the investigations to find additional mutants, and a list of aberrations and mutants found was previously reported by Sullivan & Hiroyoshi (1960). An additional list of house fly mutants is included in this report. Also, studies of the linkage relationships of these mutants and mutants obtained from other investigators are reported.

MATERIALS AND METHODS.—Examination with a dissecting microscope of adult flies from normal stocks, mutant stocks and experimental crosses has resulted in the finding of a number of morphological aberrations. Mutagenic agents were not used; only spontaneously occurring mutants were studied in this investigation. When an aberration was found, selection and brother-sister inbreeding were used in attempts to establish pure strains. The pure strains thus established were then tested genetically to determine whether the character was monofactorial. The new mutants were then used for linkage tests.

The tests to classify marker genes in linkage groups were performed by the method of F_2-test and/or testcross. These experimental crosses were performed by a single pair mating technique reported by Sullivan (1960). A batch of eggs from a single pair of flies, 100 to 200 eggs, was placed in a one-half-pint milk bottle, half-full of larval medium, which was made up in the following proportions: 1000 gr. CSMA (Chemical Specialties Manufacturers Association) dry house fly medium, 100 ml. dark Karo Syrup, ½ lb. bakers yeast and 2.5 l. water. The top of the bottle was covered with a layer of paper towel and incubated at a temperature of 80° F. for 3 days. Then about 3 tablespoons of fresh medium were added to the cultures in which the larvae were growing and the bottles were returned to the 80° F. room until emergence. A female house fly will continue to lay batches of eggs until she dies, but only two batches of eggs were kept. These egg batches were usually laid when the female was 4 to 5 and 6 to 7 days old. Only eggs from young females were used in this investigation to insure this control on crossing over rates.

Several normal or wild type house fly strains were used as sources of the new mutants and in the linkage tests. As these strains have been maintained in this laboratory for several years since being sent from other institutions and used for research on resistance to certain insecticides, some knowledge of their reactions to insecticides is available. This matter, however, seemed unimportant in this

[1] Contribution No. 1086, Department of Entomology, University of Kansas, Lawrence. This investigation was supported by the Medical Research and Development Division of the Office of the Surgeon General of the Army under Contract No. DA-49-007-MD-738. Accepted for publication May 2, 1960.

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The author thanks Dr. Robert L. Sullivan for his interest and criticism in the experiments and help in revision of the manuscript; Mrs. Elizabeth T. Lichtwardt for her encouragement during the research and for reading the manuscript, and Mrs. Mary Shepard for her technical assistance. Thanks are also given to Dr. Robert R. Sokal and Dr. Charles D. Michener for providing the opportunity to study in their laboratories.

particular report, so these wild type strains are merely referred to as normal type or the + symbol is used without reference to their reactions to insecticides.

Several excellent mutant strains obtained from other laboratories were utilized for the study of linkages, besides the mutants found in this laboratory. These mutants are listed together with our new mutant strains with citation of the original finder.

RESULTS.—Numerous morphological aberrations of the house fly were detected during the course of this study. No mutagens were utilized in this study and no prior history of the use of mutagens on the strains studied is known; therefore, all mutant types found are of spontaneous origin. During the period from April, 1958 to October, 1959 over 200 different anomalous characters affecting shape, size or color of the body, eyes, legs, bristles, wings and wing veins were recognized. Their progenies in F_1, F_2 and F_3 generations were carefully examined to determine whether these characters were heritable. Approximately 100 heritable characters were recovered. With characters of good expression the continuous pressures of selection and inbreeding were carried on in the establishment of pure strains. Many of the characters, especially various wing vein aberrations, failed to become pure, probably owing to poor penetrance in our standard culture conditions. About 50 characters were fixed in stable strains. After confirming the stability of the strains for several generations they were mated to mutant strains and the phenotypic segregations in the F_1 and F_2 progenies were studied to determine the mode of inheritance. For the mutant characters clearly inherited as single factors the mutant names were given and linkage tests were performed.

The following list includes those mutants whose linkage relationships were determined in this study. The list includes in the following order: genetic symbol, full mutant name, finder when other than the author, year of discovery if known, reference if published, source, morphological description, evaluation on expressivity and fecundity, etc.

LIST OF MUTANTS

bwb: brown-body, Sacca. Listed by Milani (1954) under the name of *yellow-body*, and the name was changed later. Body color is a yellowish-brown. An excellent recessive marker.

car: carnation, 1958. New. Derived from "orange-eyed" stock of Zingrone.[3] Eye color is pinkish-red, darkening with age without overlapping with normal type. The combination of *car* as a double recessive homozygote with *cm* gives a pale pink eye color, or, likewise, with *rb*, yellow or pale orange. An excellent recessive marker.

cm: carmine, 1958. New. Found in several males and females from a cross of *bwb* and w^{57} and later the same mutation was found from different origins. Eye color is a semi-transparent dark ruby, darkening with age but clearly distinguished from normal type. An excellent recessive marker.

cp: clump, 1958. Published by Sullivan & Hiroyoshi (1960). Found in a normal type laboratory strain. The 4th longitudinal vein is heavily thickened, forming a knot of the vein, in the posterior region of this wing vein. This thickening is usually very heavy but occasionally only a small hook of extra vein is found in the same region. Homologous characters are often found from other origins but none of them has proved to be the same genetically. Penetrance of this recessive marker is rather poor. (fig. 1, B).

ct: cut, 1958. New. Appeared as one female in an experimental culture. Wing is cut up to one-quarter of its normal length, with a uniform pattern consisting of a V- or U-shaped incision. The posterior regions of L_3 and L_4 veins and parts of the submarginal, first and second posterior cells are missing. Occasionally the heterozygote, +/*ct*, shows a slight chip in the wing tip, but is sharply differentiated from the homozygote, *ct*/*ct*. An excellent marker. Phenotypically this mutant resembles Milani's *clipped* which was sent to this laboratory and lost earlier. (figs. 1, C and C′).

ge: green, Zingrone. Published by Zingrone *et al.* (1959). Eye color varies between pale yellow-green and orange. When the larvae are raised in a mixed culture together with normal type some of *ge* individuals show intermediate eye color, pink or pale red. The genetic block at the first step of the metabolic sequence, tryptophan to brown pigment, was demonstrated in this mutant by Ward & Hammen (1957). An excellent recessive marker.

it: interrupted, 1958. Published by Sullivan & Hiroyoshi (1960). Found in an experimental culture. Vein L_4 is broken in the region where it curves upward toward the costal margin of the wing, not extending to the wing margin. Penetrance is rather poor. (fig. 1, F).

Lp: Loop, 1958. New. Found in *ct* stock. In the heterozygote an extra cross-vein arises from the posterior region of L_4 vein extending to L_3 vein and/or one or more extra anterior cross-veins in the 1st basal cell with a narrower distance between veins L_3 and L_4. In the homozygote vein L_4 is missing completely and vein L_3 is very thick, probably veins L_3 and L_4 are fused. The homozygotes are very rare and virtually sterile. Excellent as a dominant and homozygotic lethal ignoring the rare homozygote which is phenotypically distinguishable from the heterozygote. (figs. 1, D and D′).

oc: ocelliless, 1958. Published by Sullivan & Hiroyoshi (1960). Found as one male in the "orange-eyed" stock.[3] The entire ocellar triangle is absent, including the ocelli and ocellar bristles in most cases. Occasionally the ocellar triangle is present but misshaped and displaced from its medial location lying close to one eye, with fewer ocelli and ocellar bristles. Usually the distance between the eyes is wider, up to twice that of the normal type, in both sexes. A good recessive marker.

ocra: ocra, La Face. Published by Milani (1954). Eye color is pale yellow-green or yellow-orange. Under our culture conditions usually there is no phenotypic distinction from *ge*. An excellent recessive marker.

ps^{58k}*: pigmented-sternite-*58k, 1958. Published by Sullivan & Hiroyoshi (1960). One of many allelic mutants found from various origins. The sternites and back of the abdomen are blackened, especially striking in the male. The degree of blackness depends upon their origins. This *ps*^{58k} is one of the darkest strains among many *ps* mutants. An excellent recessive marker.

rb: ruby, 1958. New. Derived from the "orange-eyed" stock.[3] Eye color is bright red, paler than *cm*. The combination of *rb* as a double recessive homozygote with *car* shows yellow or pale orange eye color. A recessive marker.

ro: rough, 1958. New. Found as one male in the F_2 of a cross between w^{57} and + type. Eye surface is rough due to irregular arrangement and fusion of the facets. The expression is varied from an extreme affecting the whole eye surface to individuals in which only the periphery of the eye is roughened. An excellent recessive marker.

sb: subcostal-break, 1958. Published by Sullivan & Hiroyoshi (1960). Found from various origins. The subcostal vein of the

[3] "Orange-eyed" stock of Zingrone: Established by Miss Louise D. Zingrone, Illinois State Natural History Survey, Urbana, Illinois, from one orange-eyed fly in the field in Illinois in 1955. The stock was brought to this laboratory in 1958 and produced various eye colors, from pale greenish-yellow, orange and red to normal eye color. From this stock strain several eye color and other mutant types were separated by the author.

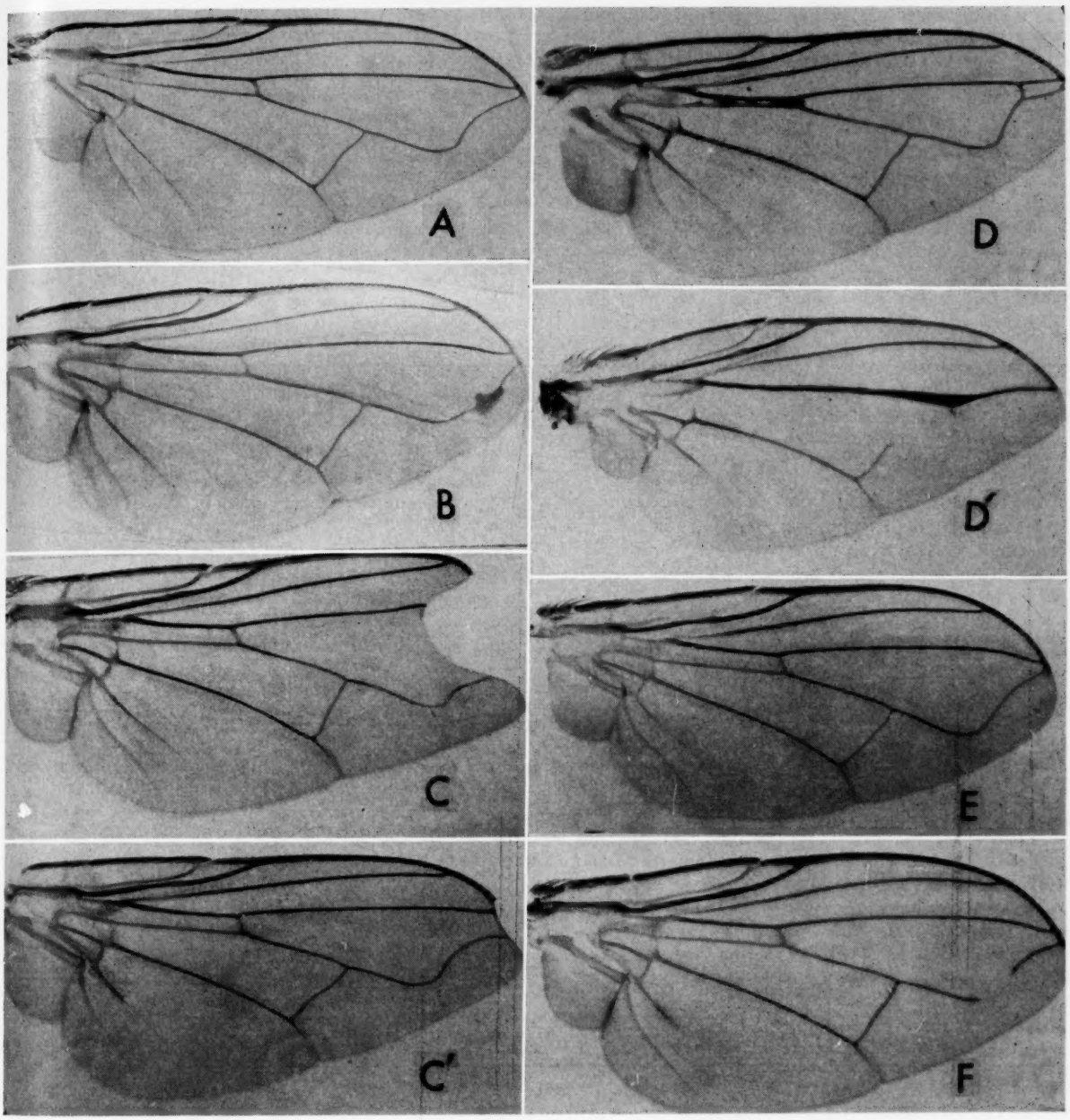

FIG. 1.—Photographs of the wings of the normal and mutant house flies: A, normal type; B, *cp* (*clump*); C, homozygous and C′, heterozygous *ct* (*cut*); D, heterozygous and D′, homozygous *Lp* (*Loop*); E, *sb* (*subcostal-break*); and F, *it* (*interrupted*).

wing is interrupted up to 2/3 of its length. Penetrance is rather poor. (fig. 1, E).

w: white, Zingrone, 1955. Sullivan (1960). Found as one fly in the field. Eye color is white. An excellent recessive marker.

w^{57}: *white-57*, Lichtwardt, 1957. Sullivan (1960). Found in a normal type laboratory stock. Eye color is white. An allele of *w*.

w^{au}: *auburn*, 1958. New. Found in the "orange-eyed" stock.[3] Eye color is dull brown, darkening with age toward the normal type but classified with a little experience. The heterozygote, w/w^{au} or w^{57}/w^{au}, shows a paler brown eye color than the homozygote of w^{au}, and no crossing over between the *w* locus has been demonstrated, probably an allele of *w*.

LINKAGE TESTS.—When our experiment to classify the mutant genes into linkage groups was begun it was known that the recessive genes for *bwb* (*brown-body*) and *ocra* (*ocra*) were not linked to each other and also that neither of these genes gave evidence of sex-linkage (Milani 1958). These two mutants had been sent to this laboratory by Milani and our test confirmed his results. The new mutants were mated with *bwb* and *ocra* and their phenotypic segregations in F_2 progenies were examined in order to find the linkage relationships of the tested markers with *bwb* and *ocra*.

The first mutant which showed no linkage relationship

Table 1.—Linkage tests between *ct* and *bwb*; *ocra* mutants and the testcross of a triple recessive mutant, *bwb*; *ocra*(*ct*).

F_2-PHENOTYPE	TYPE OF CROSS AND NUMBER OF PAIRS TESTED					
	F_2 (*bwb; ocra* ♀ × *ct* ♂): 10 & F_2 (*ct* ♀ × *bwb; ocra* ♂): 20			*bwb; ocra* (*ct*) ♀ × *bwb; ocra* (*ct*)/+ ♂: 8 & *bwb; ocra* (*ct*) ♀ × +/*bwb; ocra* (*ct*) ♂: 18		
	♂	♀	Total	♂	♀	Total
+; +; +	975	958	(1,933)	158	129	(287)
+; +; *ct*	327	304	(631)	135	123	(258)
+; *ocra*; +	309	257	(562)	137	155	(292)
+; *ocra*; *ct*	100	77	(177)	155	129	(284)
bwb; +; +	310	300	(610)	165	117	(282)
bwb; +; *ct*	108	117	(225)	157	138	(295)
bwb; *ocra*; +	89	103	(192)	140	104	(244)
bwb; *ocra*; *ct*	50	27	(77)	126	99	(225)
	(27:9:9:3:9:3:3:1)			(1:1:1:1:1:1:1:1)		
χ^2-TEST	$\chi^2=16.632$* d.f.=7			$\chi^2=16.897$* d.f.=7		

* significant at 5% level.

with either *bwb* or *ocra* was a wing shape mutant, *ct* (*cut*). Linkage tests were performed by both the F_2-test between *ct* and *bwb*; *ocra* strains and the testcross of a triple recessive mutant (the backcross of the male heterozygote between a normal type fly and the *bwb; ocra*(*ct*) to the homozygous *bwb; ocra*(*ct*) female). Table 1 shows the results of these cross experiments. In the case of the F_2-test the data fit a 27:9:9:3:9:3:3:1 ratio with a χ^2 value of 16.632 with a P between 0.02 and 0.01. From the testcross the segregation approaches a 1:1:1:1:1:1:1:1 ratio again with a P between 0.02 and 0.01. Although in both cases the χ^2 value is significant, the deviation from the expected ratios seems to be due to the reduction in the number of flies of particular mutant classes. If *ct* were linked to either of the testers the segregation in the F_2 would be 6:3:3:2:1:1 with two less classes and in the testcross a 1:1:1:1 ratio with four less classes would be expected. Therefore, the markers *ct*, *bwb* and *ocra* assort at random.

The eye color mutant *cm* (*carmine*) was tested with *bwb*, *ocra* and *ct* (table 2). The separate experiments show no evidence of linkage. The F_2 progenies between *cm* and *bwb* and also between *cm* and *ct* fit the expected 9:3:3:1 ratio and there is no doubt that *cm* is not linked to *bwb* or *ct*. The fact that *ocra* and *cm* both affect eye color leads to the problem of what the individuals homozygous for both genes would show as a phenotype. In the F_2 progenies between *ocra* and *cm* the segregation ratio very closely approximates 9 normal:3 *cm*:4 *ocra* phenotypes. Thus it appeared that among the individuals classified as *ocra* there were also flies homozygous for both the *cm* and *ocra* genes. In order to examine the genotypes of the *ocra* phenotyped F_2 flies they were mated to homozygous *cm* flies and about one-fourth of the pairs produced all *cm* phenotyped progenies, one-half of the pairs produced both *cm* and + type flies and the remaining one-fourth produced all + phenotypes. Thus it is seen that about one-fourth of the *ocra* phenotyped flies in the F_2-test were also homozygous for the *cm* gene as well as for *ocra* which masked the *cm* effect. The 9:3:4 ratio is assumed therefore to be the modified form of 9:3:3:1 ratio in this cross. The *cm* gene is therefore shown to segregate independently of the other three genes, *bwb*, *ocra* and *ct*.

The segregation ratios from all the F_2-tests of the wing vein mutant *sb* (*subcostal-break*) against *bwb*, *ocra*, *ct* and *cm* fit the expected 9:3:3:1 ratio satisfactorily and it was

Table 2.—Linkage tests between *cm* and *bwb* (second linkage group), *ocra* (third) and *ct* (fourth).

TYPE OF CROSS AND NO. OF PAIRS TESTED		F_2-SEGREGATION				χ^2-TEST[a]
		+	*cm*	*bwb*	*cm; bwb*	
F_2 (*cm* ♀ × *bwb* ♂)						(9:3:3:1)
10 Pairs	♂	970	318	265	94	
	♀	900	299	299	96	$\chi^2=5.275$ n.s.
	Total	(1,870)	(617)	(564)	(190)	d.f.=3
		+	*cm*	*ocra* and *cm; ocra*		
F_2 (*cm* ♀ × *ocra* ♂)						(9:3:3:1)
20 Pairs	♂	1,504	488	663		
	♀	1,380	440	621		$\chi^2=0.977$ n.s.
	Total	(2,884)	(928)	(1,284)		d.f.=2
		+	*cm*	*ct*	*cm; ct*	
F_2 (*cm* ♀ × *ct* ♂)						(9:3:3:1)
F_2 (*ct* ♀ × *cm* ♂)						
10 Pairs each	♂	1,023	314	340	122	
	♀	950	341	284	117	$\chi^2=3.457$ n.s.
	Total	(1,973)	(655)	(624)	(239)	d.f.=3

[a] n.s. non significant.

assumed that *sb* is independent of the other marker genes (table 3).

The independent segregation of the five mutants, *bwb* (*brown-body*), *ocra* (*ocra*), *ct* (*cut*), *cm* (*carmine*) and *sb* (*subcostal-break*) and the absence of linkage relationships between them has been shown. They are regarded as representative markers of the five autosomal linkage groups in the house fly.

The linkage relationships of the following additional mutants with these representative markers were determined. The detailed data have been omitted from this paper and only summary conclusions are included.

The mutants *cp* (*clump*), *ro* (*rough*), *ge* (*green*) and *w* (*white*) belong to the linkage group represented by *bwb*. Also the markers w^{57} (*white-57*) and w^{au} (*auburn*) which are assumed to be alleles of *w* are in this linkage group. These conclusions are based on the F_2 segregations of the mutant with *bwb* yielding a 2:1:1 ratio without a double mutant class and also on the segregations of 9:3:3:1 when tested with the markers representing linkage groups other than *bwb*.

The dominant homozygous lethal gene *Lp* (*Loop*) was found to be linked with *ocra*. The backcross of the F_1 male showing *Lp* phenotype from a cross between *Lp* and *ocra* to an *ocra* female gave approximately the same number of *ocra* and *Lp* individuals and no double mutant or wild type classes. When tested against marker genes in other linkage groups in the same manner a 1:1:1:1 ratio was obtained including the wild type and double mutant classes.

The eye color mutant *rb* (*ruby*) was found to be linked with *ct*. The results of all F_2-tests deviated significantly from expected segregation ratios, probably due to poor viability of *rb* mutant or the eyes of some prospective *rb* individuals might be normal when the larvae are reared in a mixed culture. However, no double mutant class was found in the F_2 between *rb* and *ct*, and in tests with the other markers in other linkage groups the double mutant classes were found. Therefore, the marker *rb* is assumed to be linked to *ct*.

The body color mutant ps^{58k} (*pigmented-sternite-58k*), an eye color mutant *car* (*carnation*) and *oc* (*ocelliless*) were demonstrated to belong to the linkage group represented by the *cm* gene. The results of F_2-tests with *cm* were positive and tests with other markers were negative.

Finally, the wing vein mutant *it* (*interrupted*) was demonstrated to be of the same linkage group as *sb*, again as shown by a 2:1:1 ratio in the F_2-test between them and 9:3:3:1 ratios when tested with the other tester stocks.

DISCUSSION.—A tremendous number of morphological aberrations, many of which proved to be heritable, were detected in house fly strains which had never received mutagenic treatment and probably had a tendency to be selected for homogeneity during the long period of maintenance in the laboratory. The high degree of morphological variability found in the house fly would suggest that in nature the wild type or normal form would also show high variability which would affect not only morphology, but also physiological and behavioral characteristics. The high degree of variability seems to be one of the factors that would make the house fly adaptable to the varied worldwide environments in which it is found and also might explain the rapid development of insecticide resistance in this organism.

The diploid number of chromosomes in the house fly was first shown by Stevens (1908) to be 12. This has been confirmed by several investigators and also it has been revealed that the set of chromosomes consists of five pairs of autosomes and a pair of sex chromosomes, heterozy-

Table 3.—Linkage tests between *sb* and *bwb* (second linkage group), *ocra* (third), *ct* (fourth) and *cm* (fifth).

TYPE OF CROSS AND NO. OF PAIRS TESTED		F_2-SEGREGATION				χ^2-TESTS[a]
		+	*sb*	*bwb*	*sb; bwb*	
F_2 (*sb* ♀ × *bwb* ♂) 4 Pairs	♂	944	327	318	94	(9:3:3:1)
F_2 (*bwb* ♀ × *sb* ♂) 29 Pairs	♀	955	359	275	119	χ^2=6.819 n.s.
	Total	(1,939)	(686)	(593)	(213)	d.f.=3
		+	*sb*	*ocra*	*sb; ocra*	
F_2 (*sb* ♀ × *ocra* ♂) 26 Pairs	♂	1,136	377	406	113	(9:3:3:1)
	♀	1,154	381	347	115	χ^2=2.514 n.s.
	Total	(2,290)	(758)	(753)	(228)	d.f.=3
		+	*sb*	*ct*	*sb; ct*	
F_2 (*sb* ♀ × *ct* ♂) 14 Pairs	♂	1,447	442	460	158	(9:3:3:1)
F_2 (*ct* ♀ × *sb* ♂) 30 Pairs	♀	1,381	453	455	177	χ^2=4.046 n.s.
	Total	(2,828)	(895)	(915)	(335)	d.f.=3
		+	*sb*	*cm*	*sb; cm*	
F_2 (*sb* ♀ × *cm* ♂) 10 Pairs	♂	541	168	164	54	(9:3:3:1)
F_2 (*cm* ♀ × *sb* ♂) 10 Pairs	♀	576	153	166	56	χ^2=8.076 *
	Total	(1,117)	(321)	(330)	(110)	d.f.=3

[a] n.s. non significant; * significant at 5% level.

gous in the male; that is, XY-type sex determination (Perje 1948). From this cytological evidence one would expect to find five autosomal linkage groups in the house fly. In the present study representatives of five linkage groups which behave as autosomes, lacking the typical criss-cross inheritance of sex-linked characters, have been reported. The five linkage groups described are at present named as follows, according to the order in which the first markers were found: group 2, the group containing *bwb, cp, ge, ro, w* and its alleles w^{57} and $w^{a\,u}$; group 3, *ocra* and *Lp;* group 4, *ct* and *rb;* group 5, *cm, car, oc* and ps^{58k}; group 6, *sb* and *it*. The name "Group 1" is reserved for sex-linked factors according to customary genetic usage, although they have not yet been found.

As for the sex-linked genes, although no genetic study had been made in the house fly at the time, Perje (1948) predicted that only a few or perhaps no sex-linked genes would be expected in *Musca domestica* L., for according to her cytological observation both X and Y chromosomes are heterochromatic to a great extent, and it is general knowledge in cytogenetics that phenotypically expressed genes are rare or lacking in the heterochromatic portion of chromosomes. The finding of no sex-linked gene in spite of the detection of quite a number of autosomal marker genes in our study and in other investigations would tend to verify Perje's prediction.

Sullivan (1960) has reported a mutant strain with sex-limited inheritance, which produces all *bwb* females but all normal phenotyped males. According to the genetic analysis of the strain he concluded that a part of or the entire Y-chromosome might be translocated or attached to one of the autosomes, equivalent to the second linkage group in this paper, on which the *bwb* gene is located. Recently it was found that crosses of males of several different strains to females carrying mutant genes of the second linkage group have yielded sex-limited inheritance similar to that reported by Sullivan. If his explanation for the strain is correct, the reciprocal translocation involving the Y-chromosome and autosome is very common in the house fly.

Milani (1958) has reported the occurrence of male crossing over in the house fly. He has studied the marker genes in the second linkage group and reported high male crossing over values, for instance, as high as 1.65% between *bwb* and *div* (*divergent*) and 2.56% between *div* and *kdr* (*knockdown-resistance*) and so on. Sullivan (1960) has also recognized male crossing over in studies with genes in the same linkage group, although the values are low compared with those of Milani, 0.11% between *bwb* and *ge* and 0.03% between *bwb* and *w* in male flies. These crossing over values in male flies are based on the number of recombinants from the backcross of heterozygous males for two recessive genes to the homozygous females. Contrary to these results, no recombinants from the similar kind of cross experiments conducted with some genes in the second linkage group, the same group they studied, and also with genes in the third and fifth linkage groups have been detected so far by the author. Moreover, among many experiments, no fly in the double mutant class from F_2-tests between two recessive genes has been detected when the two genes are in the same linkage group. However, these results do not disprove male crossing over in the house fly in general but only suggest that usually there is no crossing over in the male or at least the frequency is very low, and those detected by Milani and Sullivan might be exceptional cases. Furthermore, the phenotypic recombination found from the testcross of males does not necessarily mean the occurrence of male crossing over but can be explained by other means, for instance, the elimination of a gene or chromosome and the recovery of the gene effect which was not expected under normal circumstances. The frequent occurrence of mosaic and intersex individuals in the house fly, as was observed both by Milani and Sullivan, suggests the possibility of gene or chromosome elimination in high frequency. At any rate, to confirm whether the crossing over in the male fly occurs very commonly or not and how frequently it occurs is very important to promote the genetics of this insect and more precise study on this subject is necessary from the viewpoints of cytology and genetics.

At present a simple linkage map of the house fly is being completed with mutants described in this paper and some others, and this will be reported soon. For the investigator's convenience several excellent multichromosomal mutants, such as *ro; ct; cm; sb* (2; 4; 5; 6) and *bwb; ocra; ct;* ps^{58k}; *sb* (2; 3; 4; 5; 6) strains etc. and also strains with more than two mutant genes on a chromosome, such as *bwb, ge, ro* (2) and *cm, car, oc,* ps^{58k} (5) etc. have been synthesized and are being maintained in the Department of Entomology, University of Kansas.

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Laboratory Tests of Insecticides Against Eggs and Larvae of the Cabbage Looper[1]

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ABSTRACT

Laboratory tests were conducted on laboratory-reared cabbage loopers (*Trichoplusia ni* (Hbn.)) to determine the toxicity of six insecticides applied at three rates to eggs and various larval instars. Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) was the most effective ovicide. All doses of Sevin® (1-naphthyl methylcarbamate) and 2 pounds of DDT per acre were moderately effective on eggs. First instar larvae were relatively susceptible to all of the insecticides but endrin and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) produced extremely high mortality, while Phosdrin and Sevin were the least effective. Larvae in the second and the third instars were progressively less susceptible to the insecticides tested. Endrin and Thiodan were the most effective materials used for control of larvae through the third instar. None of the insecticides was effective against larvae in the fourth and fifth instars. For effective control of the cabbage looper it will be necessary to apply insecticides before the larvae attain the fourth instar.

The cabbage looper, *Trichoplusia ni* (Hbn.), has become a serious pest of cotton in the southwestern United States during the last 10 years. Generally the larvae feed on the older leaves in the central and lower portions of the cotton plant causing a ragging appearance. However, in case of severe infestations the plants may be defoliated resulting in yield losses.

Naturally occurring epizootics of a polyhedral virus disease frequently "wipe out" cabbage looper infestations. However, the disease cannot be relied upon to occur in all cases and usually does not become effective before severe infestations have developed. This results in a need for frequent insecticidal applications for the control of the pest.

Excellent control of the cabbage looper was obtained with DDT for several years after the use of this material as an insecticide, Smith & Harrison (1944), Apple (1945), Huckett (1946) and others. However, by 1954 Wilcox and Howland reported difficulty in controlling cabbage looper larvae with DDT in California; also, McEwen & Hervey (1956) reported unsatisfactory results in New York. These results and further field observations indicated that the cabbage looper had developed resistance to DDT. Because certain insecticides seemed to have a suppressing effect on cabbage looper populations, it appeared likely that some insecticides were effective in killing the earlier larval instars but not on larger larvae.

METHODS.—A laboratory culture of the cabbage looper was maintained to provide test insects. The adults used for egg production were collected from a light trap as needed to maintain the culture. The adults were placed in a 1-quart ice cream carton lined with colored blotter paper for oviposition. The moths were fed upon a mixture of honey-water (1:1). Eggs were deposited on the blotter-paper liners in the ice cream cartons and removed daily to rearing cages. One-quart ice cream cartons vented with 60-mesh screen were used for rearing cages.

Larvae were reared in a room in which the temperature was approximately at 80° F. and relative humidity of 70% to 80%. The eggs were allowed to incubate for 2 days and then placed in vented ice cream cartons with fresh cotton terminals. After hatching, the larvae moved from the blotter paper to the cotton terminals and started feeding. Every 2 days thereafter it was necessary to transfer the larvae to fresh food. As the larvae increased in size, the number of larvae per carton was decreased to prevent the spread of disease and to insure an adequate food supply.

The toxicity of six insecticides to cabbage looper including separately the eggs, first three larval instars and a mixture of the fourth and fifth instars was obtained. The insecticides used in these tests included Phosdrin®(1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), Sevin® (1-naphthyl methylcarbamate), DDT, toxaphene-DDT (2:1), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and endrin. These materials have all been used in the field with varying degrees of success for control of the cabbage looper. The three dosage levels selected for each material include the heavy, light, and medium dose of each material that would normally be applied for control in the field.

The insecticides were applied in water dilutions of commercially available emulsifiable concentrates at a rate of 7½ gallons of total mixture per acre. The material was sprayed on prepared cotton terminals in a cylindrical spray tower at a pressure of 40 p.s.i. The desired dosages were obtained by varying the dilutions so that different rates of technical material were delivered in the same amount of solution. The plants were held in the spray tower for 2 minutes after the insecticide application.

In the test with cabbage looper eggs, 1-day-old eggs were transferred to the upper surface of cotton terminals which were then treated and caged in the holding room. Ten eggs were used for each insecticide rate and replicated 8 to 16 times. Untreated checks were maintained with all tests. Mortality records were made after 48 hours and corrected for natural mortality by Abbott's formula (1925). The eggs were examined under a microscope to determine if they had hatched or if the developing embryo had died. Only mortality that occurred in the egg stage was considered ovicidal action.

In the tests with cabbage looper larvae, the terminals were treated before the larvae were released on them. Ten larvae of the proper instar were caged on a treated terminal held in a vented 1-quart ice cream carton. Each treatment was replicated 4 to 12 times. The test insects were

[1] Technical contribution no. 3518 Texas Agricultural Experiment Station in cooperation with the Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture. Accepted for publication May 5, 1960.

Table 1.—Mortality of eggs and larval instars of the cabbage looper with several insecticides.

Insecticide	Dosage (lb./a.)	Per Cent Control				
		Eggs	First Instar	Second Instar	Third Instar	Fourth and Fifth Instar
Phosdrin	0.25	76	49	30	60	26
	.50	79	64	54	76	10
	1.0	92	88	89	48	25
Sevin	0.5	59	58	38	30	25
	1.0	51	50	47	46	20
	2.0	73	68	52	20	16
DDT	1.0	19	78	37	31	22
	1.5	39	86	59	54	12
	2.0	71	96	75	83	35
Toxaphene-DDT (2:1)	0.8	3	71	66	71	2
	1.5	12	79	62	60	11
	3.0	38	88	79	65	30
Thiodan	0.50	14	85	69	67	16
	1.0	9	98	89	71	20
	2.0	9	100	100	91	16
Endrin	0.25	2	97	76	43	17
	.50	5	100	96	69	14
	.75	11	100	94	96	19

held at 80° to 85° F. in a room provided with a fan for air circulation. Untreated checks were maintained with all tests. Mortality records were made after 48 hours according to Abbott's formula (1925). Larvae were considered dead if no movement occurred when touched.

RESULTS AND DISCUSSION.—Results of the tests with six insecticides for control of eggs and larvae of the cabbage looper are given in table 1. Phosdrin was the most effective material as an ovicide, producing 76% to 92% mortality at the doses tested. A mortality range of 51% to 73% resulted among eggs treated with Sevin. Egg mortality with all other materials averaged below 50% except for the 2-pound rate of DDT. These results indicate that economical control of the cabbage looper may result when Phosdrin is used as an ovicide provided the applications are properly timed.

All materials except Sevin and Phosdrin at the two smaller doses gave more than 70% control of first instar larvae. Best control of the first larval stage was obtained with Thiodan and endrin, which proved least effective as ovicides. Also the two materials most effective as ovicides, Phosdrin and Sevin, were least effective as larvicides.

The same pattern of relative effectiveness of the insecticides was evident in tests with second and third instar larvae. However, there was evidence of a general decrease in the effectiveness of the materials as the larvae increased in size. Thiodan and endrin at higher dosages were the most effective materials for control of third instar larvae. Toxaphene-DDT-(2:1) was less effective than either Thiodan or endrin but was quite consistent in control of the three instars at each dosage. DDT was effective for third instar larvae only at the 2 pounds per acre dose.

The insecticides included in the tests failed to effect adequate control of fourth and fifth instar larvae. These data indicate clearly that cabbage looper larvae became less susceptible to the insecticides tested with each succeeding instar. These results are in accord with those of various workers who found that the bollworm (*Heliothis zea* (Boddie)) is extremely difficult to control in the later instars. Also results of the present studies indicate that when a cabbage looper population is in an early stage of development applications of appropriate insecticides would effectively control the pest. Most of the insecticides used in cotton insect control exert a suppressing effect on the cabbage looper population by killing many of the first three instars. Since some of these materials are not highly effective for cabbage looper control, there is a gradual buildup of the population to the point that ragging of the leaves becomes noticeable when they are used. This does not usually occur until a large portion of the larval population is in the fourth and fifth instars. At this time, most growers apply insecticides specifically for control of this insect and do not obtain satisfactory control.

These results indicate that control measures for cabbage loopers must be initiated while the larvae are in the first three larval instars.

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Estimation of Total Honey Bee Populations Using a Known Population of Marked Bees[1]

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ABSTRACT

A sampling method for estimating unknown honey bee populations (*Apis mellifera* L.) is proposed by using a formula developed by Ricker, $P=NH/R$, where N is a known marked bee population, H is an adequate sample of foraging bees, and R is the number of marked bees in the sample of collected foraging bees. In this trial there is estimated to be between 15,500 and 23,300 bees per colony.

Populations of honey bees (*Apis mellifera* L.) foraging in fields present an investigator with the problem of how many colonies are present. Conversely, if a given number of colonies are placed in an area, he may need to know how many foragers can be expected in the field. The problem is intensified when the colonies placed in the field contain different numbers of bees, when wild colonies are present in the woods nearby, and when other beekeepers have apiaries within a flight range of 2 to 3 miles.

At present there is no working definition of a colony that conveys the same information to both the beekeeper and the seed grower. The grower wants sufficient bees to set the seed, and the beekeeper wants compensation in either rent for bee services or sufficient honey to justify the location of the apiary.

Recent literature shows the general vagueness in determining populations of pollinating honey bees. Blake (1958) reported bee colonies at concentrations of $\frac{2}{3}$, 2, and $5\frac{1}{3}$ per acre. According to Walstrom (1958), "Colonies with strengths of eight or more frames of brood were provided at blooming time at the rate of two per acre." In estimating the effect of low populations on pollination of white clover in Mississippi, Green (1957) reported seven average colonies and he thought that about six or eight other colonies were in bee trees within a foraging area of several thousand acres, including 400 acres of white clover grown for seed. Since colony populations vary from 5 to 60 thousand bees, the number of colonies per acre does not give any idea of the total population. Furthermore, the effective field force supplied by them increases with the population throughout the population range (Farrar 1937).

A method has been developed for estimating colony populations based on field sampling of foraging bees. It is possible to obtain a fairly reliable estimate of the colonies placed for pollination by using a small number of genetically marked colonies of known population and then sampling the foraging bees over the field. The population (P) of colonies used to pollinate the crop is then calculated by the formula $P=N\times H/R$ developed by Ricker (1948) for estimating fish populations, where N is the total number of marked bees, H the total number in the sample, and R the number of marked bees in the sample. The average strength of the colonies placed in the field for pollination plus all others is estimated by dividing the calculated population by the number of colonies.

The bees may be caught, killed, and counted, or the observer may walk through the field counting the marked and unmarked bees as he goes. Peer (1956) has shown no difference between the two sampling methods. In the study reported herein the counts were made in the field, since a larger sample can be obtained over the entire field in the same time.

A number of conditions must be met in order to use this method of estimation. They are similar to those described by Scattergood (1953) for application to fish and wildlife surveys. First, the marked colonies must be in normal balance—there should be nearly equal numbers of bees of all ages and balanced brood rearing. Colonies going through a rapid buildup period when large quantities of brood and young bees are present and those that have produced a honey crop and have had their brood nest restricted should be avoided. Second, the foraging bees from the colonies containing the marked bees should be randomly mixed with the unmarked foraging bees. Third, samples should be taken from all parts of the field. Fourth, forage for the marked bees should be the same before and after the move to the experimental field.

The reasons for most of these conditions are obvious. Pollen-trap records indicate that colonies side by side frequently have their field forces foraging on different plants in different areas. Oettingen-Spielberg (1949) and von Frisch (1955) have shown that bees communicate the location of food supplies to their sisters by means of dances. If most of the bees in one hive work in one or two areas that are out of the experimental area, the best way of overcoming this source of bias is to have a large number of marked colonies. Levin & Bohart (1957) have shown that colonies moved to alfalfa from alfalfa-free locations collect about twice as high a percentage of alfalfa pollen as those already on alfalfa forage. This bias might affect the proportion of marked to unmarked bees on other crops as well.

METHOD.—Eleven small colonies of cordovan bees were used in this experiment. Artificially inseminated cordovan queens were used to insure all-cordovan populations. Two days before the colonies were moved to the test area their populations were determined by weighing them and obtaining the weight per bee from a small sample of know numbers (table 1). In behavior studies Taber (1954) and Peer (1956) found the behavior of cordovan marked bees normal.

The experimental field, consisting of 30 acres of white clover, was located 25 miles northeast of Baton Rouge. Thirty hives were placed in the field by a commercial beekeeper for pollination purposes on March 20, 1958. Some of the hives had bees covering 10 standard frames, and the stronger ones had bees covering 30. The field was enclosed on three sides by woods known to contain some colonies in trees. There were no known concentrations of bees in apiaries close enough to affect this study. Before the cordovan colonies were moved, a thorough examination of bees both on the field and in the commercial colo-

[1] Accepted for publication May 6, 1960.

[2] In cooperation with Louisiana State University.

Table 1.—Populations of 11 colonies of honey bees before move to test field.

Colony	Weight of Bees (grams)		Number of Bees		Colony Population
	Total	Sample	In Sample	Per Gram	
1	1,724	36.8	320	8.7	15,000
2	1,361	25.1	194	7.7	10,500
3	953	26.3	216	8.2	7,800
4	1,270	35.8	294	8.2	10,400
5	1,452	39.6	287	7.2	10,500
6	1,270	33.4	257	7.7	9,800
7	1,225	32.5	253	7.8	9,500
8	1,406	33.9	262	7.7	10,900
9	1,270	34.8	258	7.4	9,400
10	952	38.0	253	6.7	6,300
11	1,270	29.7	218	7.3	9,300
Total					109,500[a]

[a] For purposes of calculation this total was rounded to 110,000.

nies revealed that no cordovan bees were present. Eleven days elapsed from the time the cordovan-colony populations were determined until the last field-sample count was made. It is unlikely that the populations of these colonies changed at a substantially different rate than the commercial colonies during this time. It is, of course, not known what proportion of the cordovan population was engaged in field activities, but it seems safe to assume that there was no bias in age grouping over the 11-day period and that all samples were representative.

Both the test and commercial colonies were placed in the center of the clover field. Four samples with about 10 subsamples of 100 bees each were observed within randomized areas throughout the field to determine the number of marked and unmarked bees. Thus the field sample for each date represented approximately 1000 bees.

Results.—The sample counts and estimates of the bee populations in the experimental field are given in table 2, and data for the sample that showed the greatest subsample variation in table 3. The highly significant differences between subsamples indicate that the marked bees were not uniformly distributed over the field. It is presumed that distribution of bees from the commercial colonies was similar.

The first count was made on May 18, when no cordovan bees were found more than 100 yards from the hive. However, cloudy and rainy weather prevented much flight from the time the colonies were moved to the field. Two counts were made on May 25. The first sampled the whole field and showed 17.5% cordovan; in the second count of 350 bees made within 50 yards of the apiary 15% were

Table 2.—Honey bee populations in the experimental field.

Date	Total Number in Sample	Per Cent Cordovan	Estimated Populations per Colony[a]
May 20	849	19.2	15,500
21	1,020	15.0	20,800
22	1,020	13.6	23,300

[a] Minus cordovan bees taken to the area.

Table 3.—Analysis of May 25 sample count showing uneven distribution of cordovan honey bees.

Subsample (in Order Taken)	Total	Per Cent Cordovan
1	100	17.0
2	110	3.6
3	97	7.2
4	93	25.8
5	100	44.0
6	102	28.4
7	98	12.2
8	100	7.0
9	100	14.0
10	105	17.1
Total or average	1,005	17.5

d.f. = 9. $\chi^2 = 81.40$.

cordovan indicating there was no undue concentration of marked bees close by.

Discussion.—The density of foraging honey bees depends on the number and condition of colonies, the floral supply and its attractiveness, and the competition with all other species of nectar and pollen gatherers. If competition is severe or if the supply or attractiveness of the crop is low, honey bees may be expected to range 3 to 5 miles from their home location. On the other hand, if there is ample nectar and pollen, they are not likely to search more than 1 or 2 miles. Peer (1955) has shown that the probability of finding a marked bee during the honey flow decreases sharply beyond 2 miles. These factors that affect bee visitation on the field vary from hour to hour. However, it is possible to get different populations on different days, especially if there are apiaries containing many bees within 1½ to 3 miles. In this study no such concentrations of colonies were present within a 3-mile radius.

Usually no attempt is made to determine or standardize the populations of colonies used for pollination. The estimated 15,500 to 23,300 bees per colony determined under the conditions of the experiment indicate the general strength of the 30 commercial colonies plus all others with the approximate error of magnitude. The large chi-square shown in table 3 for concentrations of marked bees at various points in the field confirms the assumption of Oettingen-Spielberg and von Frisch that scout bees communicate the location of food sources to their sisters. Their theory implies areas or islands of concentration for each colony within a homogenous area.

The sampling method described herein provides a means for estimating the populations of a large number of colonies used for pollination by applying Ricker's formula in which a small number of genetically marked bee colonies are incorporated. It takes into account but does not separate the bees supplied by wild colonies and small apiaries located some distance away. However, it provides a more accurate estimate of their strength than the loosely used term "colony."

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Studies on the Relation Between Silica in Wheat Plants and Resistance to Hessian Fly Attack[1]

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ABSTRACT

The leaf sheaths of certain wheat plants and one variety of oats that are resistant to hessian fly attack appear to have a much more complete and even distribution of silica deposits on their surface than do those of susceptible varieties. The silica deposits in the latter varieties are in the form of rod-shaped masses which are arranged in dispersed rows. The density of deposition depends on the physiological age of the plant and emphasizes the necessity of considering this factor in studying the problem of resistance. Certain varieties were demonstrated to have silicified hairs on the surface of the sheath. Most of these varieties were susceptible to hessian fly attack.

These results suggest that the presence of silica depositions on wheat plant tissue may be one of the factors related to resistance of certain varieties to hessian fly attack. Since there are several parental sources of resistance to hessian fly other factors also may be involved.

Some have postulated that the silica content of wheat stem tissue is related to the stiffness of the stems and perhaps to resistance to attack by hessian fly (*Phytophaga destructor* (Say)). Enock (1891) stated that wheat stems containing a large amount of silica were not injured severely by larvae. McColloch & Salmon (1923) found that several susceptible varieties developed marked resistance when they were grown in a solution containing sodium silicate. The degree of resistance increased as the amount of silicate was increased in the nutrient solution. Painter (1951) considered that the amount of silica is related to resistance because it strengthens the cell walls of the plant. He also suggested that silica compounds might absorb digestive enzymes from the insect. Refai *et al.* (1955) measured the total amount of silica in the lower stem portion of wheat plants representing a wide range of resistance to hessian fly. They found no significant correlation between amount of silica and degree of fly resistance; however, one very resistant variety, Pawnee×PI 94587, did have a consistently high level of silica when grown at several locations.

Table 1.—Wheat varieties differentiated by field performance according to their degree of resistance.

DEGREE OF RESISTANCE[a]	VARIETY	
	Winter Wheats	SPRING WHEATS
1	ChiefKan—Hard Federation—Kawvale×PI 119344, Kansas Sel. 52400 Pawnee×PI 119358, Kansas Sel. 52381	Durum, PI 94587
2		Thatcher×Triunfo, CI 12497 Illinois No. 1 W38, CI 12061
3	Marquillo-Oro×Triunfo, CI 12858	
4	Kawvale, CI 8180	
S	Cheyenne, CI 8885 Malakof, CI 4898 Tenmarq, CI 6936 Oro, CI 8220	Ceres, CI 6900 Rival, CI 11708 Hope, CI 8178

[a] Complete resistance to hessian fly attack is indicated by the number 1; progressively less resistance is indicated by higher numbers; the letter *S* indicates susceptibility to hessian fly attack.

The objective of the present investigation was to study the relationship between distribution of silica in the sheath tissue of several varieties of wheat plants and for one variety of oats and resistance of the plants to hessian fly.

MATERIALS AND METHODS.—Wheat Varieties Used: The wheat varieties used in this study are listed in table 1. The Cimarron variety of oats, which is not attacked by hessian fly, also is included.

[1] Cooperative investigations between the Crops and Entomology Research Divisions, Agric. Res. Serv., U.S. Department of Agriculture, and the Kansas Agricultural Experiment Station, Manhattan. Contribution No. 317, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. This work was supported by a grant from the National Science Foundation. Accepted for publication May 9, 1960.

The plants were grown in 6-inch pots in the greenhouse. Three plants of each variety were dug at the three-leaf stage, wrapped in dampened paper toweling, placed in a plastic bag, and stored for a few hours or days at 5° C. until the spodograms were prepared.

Spodogram Technic: The preparations of ash from the stem portion of the plants (spodograms) were prepared according to the technic of Lanning *et al.* (1958). The second leaf sheath which covers the hessian fly larvae in most cases was selected for study (Esau 1953). The sec-

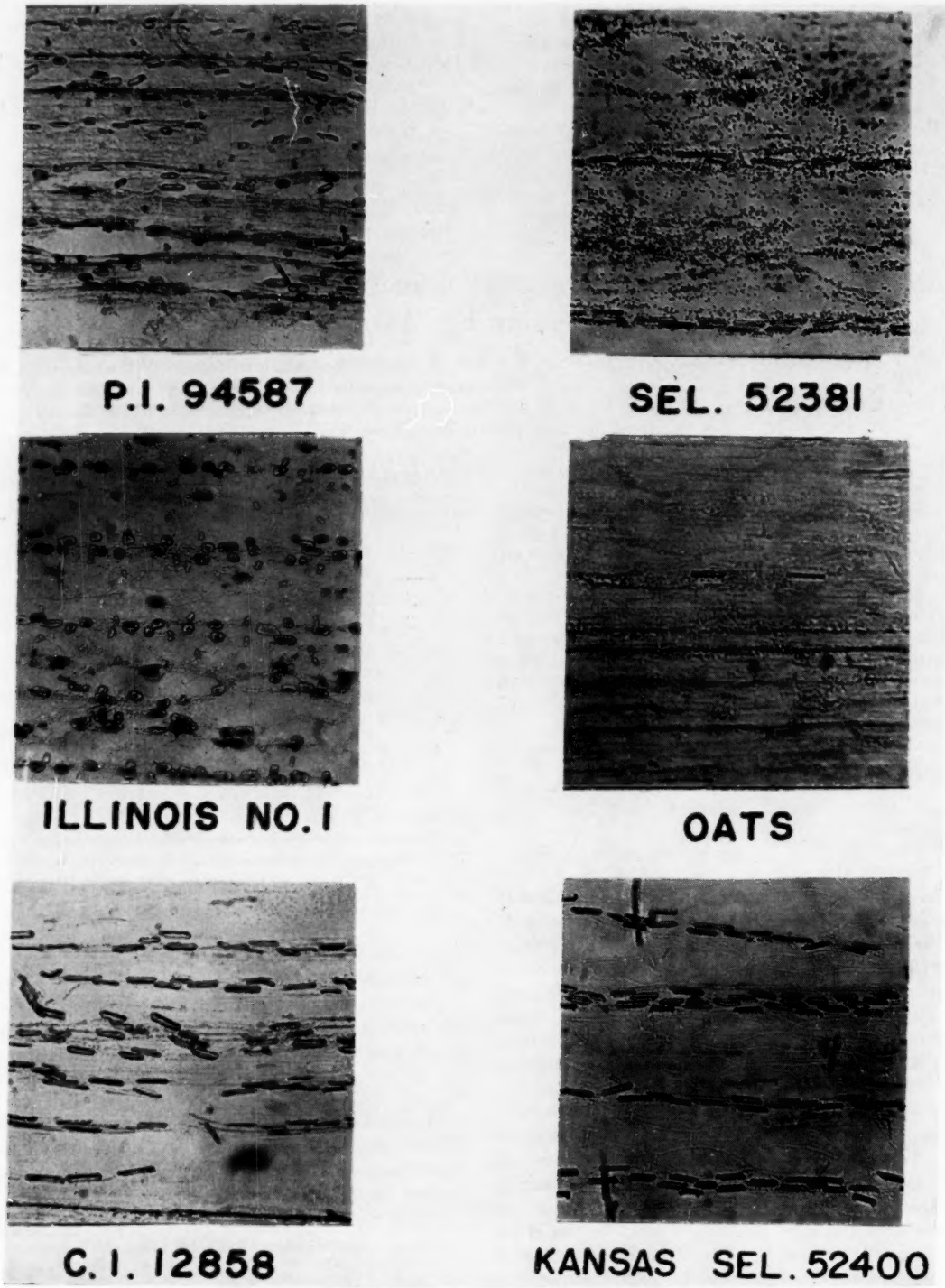

FIG. 1.—Spodograms of oats and several wheat varieties that are resistant or semi-resistant to hessian fly.

tion employed was the $\frac{3}{4}$-inch part immediately above the root system.

RESULTS AND DISCUSSION.—Spodograms for the Cimarron variety of oats and five hessian fly-resistant or semi-resistant wheat varieties representing the hard red spring, hard red winter, and the durum classes are shown in figure 1. The dark shapes of silica depositions ranging from round to oblong are quite different for these varieties. Most of these varieties have a relatively dense and grainy covering of silica over the entire surface of the sheath. This is particularly true for oats and for Illinois No. 1, PI 94587, and Kansas Sel. 52381. The pattern for silica deposition in the Varieties CI 12858 and Kansas Sel. 52400 does not appear to be the same as for the other resistant varieties. CI 12858 has a resistance rating of only 3, while Kansas Sel. 52400 has a rating of 1. Perhaps the rather faint background pattern for Sel. 52400 is also a silica deposit.

The high concentration of silica in Pawnee×PI 94587, reported by Refai *et al.* (1955) corroborates the present data. Since the other resistant varieties studied by Refai *et al.* (1955) were not particularly high in silica content, the physical arrangement of silica along the abaxial portions of the sheath may also be important. It should be recognized also that silica deposition may be only one factor related to the resistance of wheat to hessian fly. As an example of another factor which may cause resistance, Refai *et al.* (1955) have shown that the degree of toughness of wheat stem tissue was correlated positively with degree of hessian fly resistance.

The spodograms for susceptible varieties shown in figure 2 reveal that silica is deposited in rod-shaped masses arranged in rows with much space between the rows. This arrangement may allow the hessian fly larvae to feed between the rows of silica. In the case of some resistant varieties (fig. 1) there may not be enough free space to permit unrestricted feeding by the larvae.

The spodogram for the slightly resistant variety Kawvale in the upper left corner of figure 2 was prepared from a leaf sheath that was several days younger than that

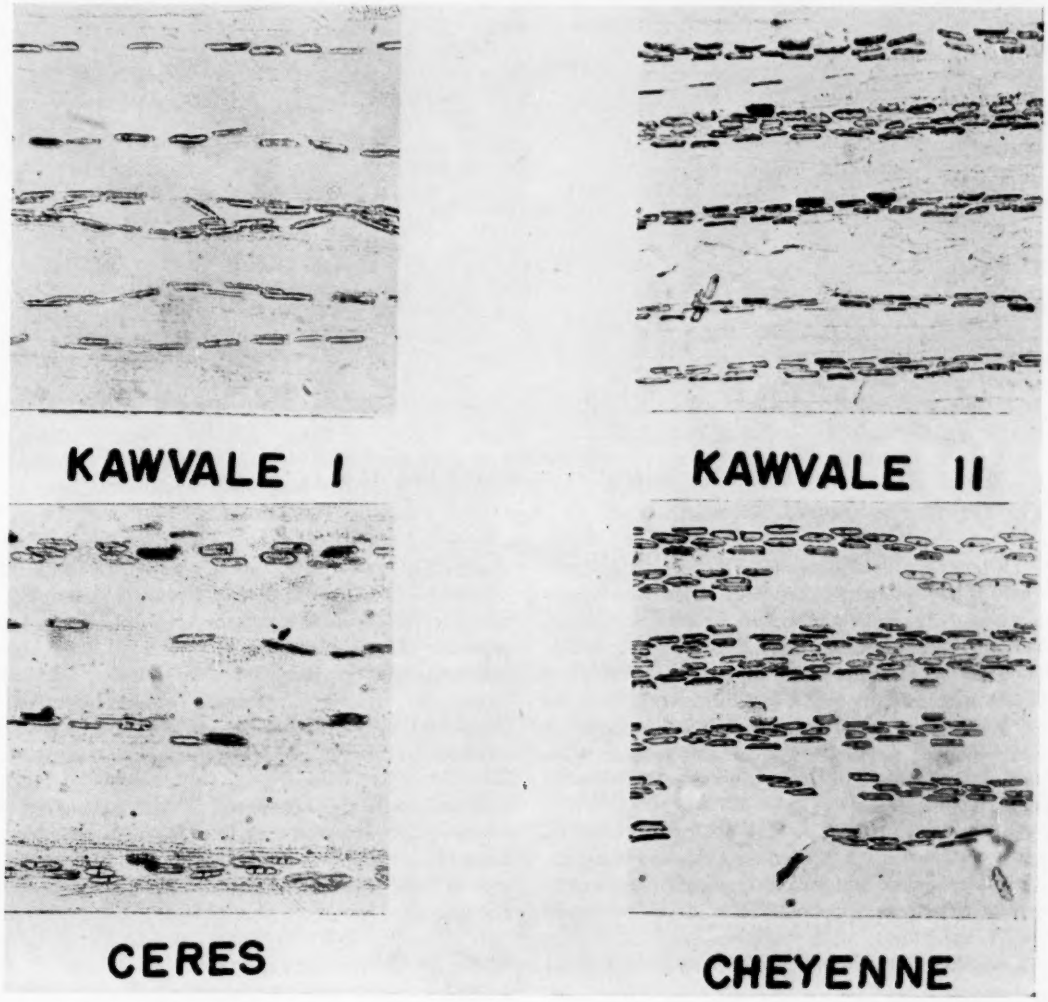

FIG. 2.—Spodograms of several wheat varieties which are susceptible or only slightly resistant to hessian fly. Kawvale II represents a spodogram that was made from plant material that was several days older than that represented by Kawvale I.

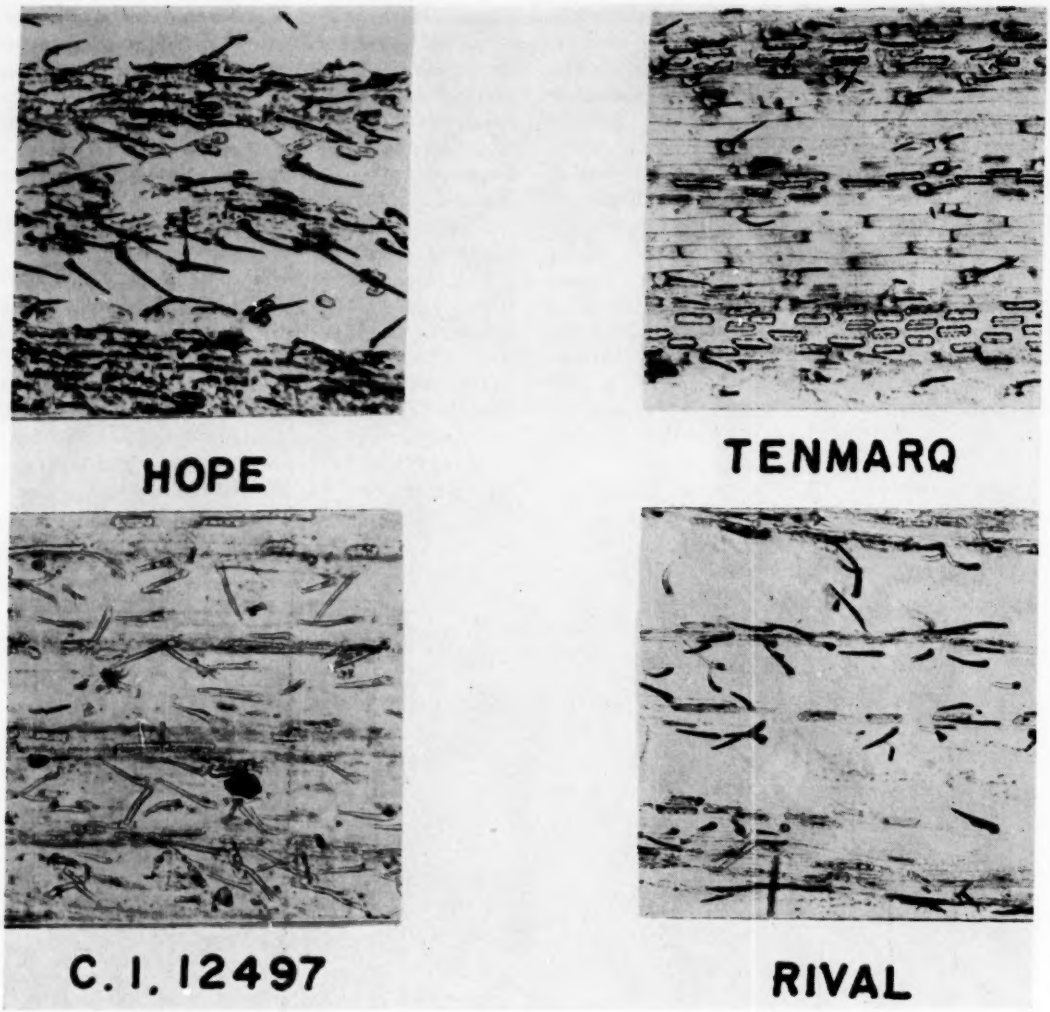

FIG. 3.—Spodograms of wheat plants which have silicified trichomes.

used for the comparable spodogram shown in the upper right corner. This illustrates the more extensive deposition of silica as the age of the plant increases and emphasizes the importance of comparing plants at comparable physiological ages. The fact that the deposition of silica on Cheyenne wheat plant tissue is more dense than on other susceptible varieties in figure 2 may be owing to its greater physiological age at the time of sampling. Spodograms made using Comanche and Malakof wheat sheaths were similar to that for Kawvale 1.

This work corroborates the findings of Volk *et al.* (1958) who worked with rice plants. The data may explain why infestation occurs only in younger plants even in susceptible varieties. Apparently the larvae prefer to feed through the young, soft, and less silicified tissue. This tendency for many insects to feed on young plant tissue is common.

Four wheat varieties were found to have developed silicified hairs, some of which appear to be hollow (fig. 3). They are particularly abundant in the Hope wheat variety. In each case oblong silica deposits also were produced. This is the first record, known to the authors, of silicified hairs in wheat plants. Rival, Hope, and Tenmarq are susceptible varieties, while CI 21497 is resistant. The presence of hairs may not be associated with susceptibility, although the hairs may cause more free space between the various leaf sheaths. However, an earlier attempt to relate the differences in intersheath space among varieties to different degrees of resistance was not successful.

Spodograms were prepared for several barley varieties representing different degrees of resistance. No conclusions concerning the relationship of silica deposits to degree of resistance could be drawn from these data. Most of the deposits, however, were oblong in shape.

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Biology and Control of the Little House Fly, *Fannia canicularis*, in Massachusetts[1]

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ABSTRACT

The little house fly (*Fannia canicularis* (L.)) has become a serious annoyance problem on many Massachusetts poultry farms. In the laboratory, the larvae were reared in a modified Chemical Specialties Manufacturers Association (C.S.M.A.) medium. At an air temperature of 80° F., the approximate developmental periods for the immature stages were as follows: egg 1½ to 2 days, larva 8 to 10 days and pupa 9 to 10 days. The total developmental period from egg to adult was 18½ to 22 days and from egg to egg, 22 to 27 days. The sex ratio of emerging adults was approximately 0.5. At 80° F. and 65% relative humidity, 50% of the male population died within 14 days after emergence, with some living up to 28 days, while 50% of the female population died within 24 days with a few living for 54 days. Chicken manure was a preferred oviposition medium. Adults were unable to fly against winds that exceeded 16 m.p.h

In the field, more than one-half of the poultry farms inspected employed the pit system of droppings management and these farms had heavier fly populations than farms which effectively employed the open floor system. Observations on the distribution of overwintering larvae in the dropping pits are presented. All stages except the egg were found during the winter months. Adults became active in early March and were present in large numbers throughout the summer until mid-October. Adults were also present in homes near poultry farms except during the month of February. A preference of adults for cooler areas under 84° F. was apparent. Field recognition characters of the adult and larvae are discussed.

The use of parathion-Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) treated cords gave excellent seasonal control of the little house fly but supplemental treatments with baits were necessary against *Musca domestica* L.

The annoyance caused by a heavy fly population is sometimes a serious problem. Complaints by Massachusetts residents and poultrymen regarding the little house fly, *Fannia canicularis* (L.), have increased recently, especially in the early spring. The problem has been aggravated by rapidly expanding residential sections around poultry farms. A preliminary survey revealed that this situation was common, and that it had resulted in action by local public health officials in some towns.

In addition to the concern created by this fly because of its presence in large numbers, its disease-producing potentialities have also been noted. Hewitt (1912) reported this species as a causative agent of intestinal and urinary myiasis, and Lochhead (1919) reported it as a carrier of the typhoid bacillus. Also Burnett *et al.* (1957) reported developmental stages of the eye worm, *Thelazia californiensis* Price, in *F. canicularis* adults, and suspected it as an intermediate host of this parasite, the definitive hosts being man, cat, dog, deer and other mammals in the Pacific Northwest.

REARING PROCEDURES.—Satisfactory collecting of adults in the field to initiate a laboratory culture was accomplished with an electrically operated aspirator which drew resting adults into pint paper cartons over which screened covers were placed.

The adults were transferred into holding cages and fed two separate solutions, one of household molasses and the other canned evaporated milk. Both were diluted with equal parts of water. These solutions were afforded in 5-ounce paper cups, each containing a piece of crumpled paper towelling which served as an alighting surface for the feeding adults. These food sources were replaced twice weekly.

Although eggs were laid on the crumpled paper towelling in the milk containers, a more satisfactory oviposition site was derived by the addition of household ammonia diluted with equal amounts of water to dry Chemical Specialties Manufacturers Association (C.S.M.A.) medium.[3]

The larval rearing medium was formulated by the addition of a 20% molasses solution, containing one yeast cake per 1,000 ml., to the dry C.S.M.A. mix to just under saturation. The ammoniated medium containing the eggs was removed from the adult holding cages and used as inoculum to initiate larval populations. Placement of 1½

[1] Contribution No. 1240 of the Massachusetts Agricultural Experiment Station, Hatch No. 166, and Entomological Contribution No. 1331 from the Department of Entomology and Plant Pathology of the University of Massachusetts.

Portions of a thesis by the author submitted to the faculty of the University of Massachusetts in partial fulfillment of the requirement for the Degree of Master of Science in Entomology and supported by the U. S. Public Health Service. Accepted for publication July 5, 1960.

[2] The author wishes to thank Dr. H. L. Sweetman, Dr. F. R. Shaw and Dr. J. H. Lilly for their valuable assistance and counsel.

[3] A prepared cereal mix available from Ralston Purina Co., St. Louis, Missouri.

inches of the larval medium in battery jars of 6-inch diameter was ample for rearing approximately 100 larvae. The jars were then placed in an 80° F. temperature chamber where the humidity was maintained at approximately 65%. Pupae were obtained by placing tightly rolled cardboard on the surface of the medium or by washing the medium through a cone-shaped kitchen strainer, so as to leave the pupae behind.

LIFE HISTORY.—At an air temperature of 80° F., the approximate developmental periods for the immature stages were as follows: egg 1½ to 2 days, larva 8 to 10 days and pupa 9 to 10 days. The preoviposition period was 4 to 5 days. Thus the total developmental period from egg to adult was 18½ to 22 days and from egg to egg, 22 to 27 days.

The ranges and average lengths of the immature stages in mm. were as follows: egg 0.80–0.95, avg. 0.88; first instar 0.80–1.10, avg. 1.00; second instar 2.60–3.20, avg. 2.80; and third instar 5.80–7.50, avg. 7.00. These data are comparable with those of Lewallen (1954), with the exception of the size of the egg which he reported as being 2.0 mm.

The sex ratio of emerging adults was approximately 0.5. At 80° F. and 65% R. H., 50% of the male population died within 14 days after emergence, with some living up to 28 days. Under these conditions, 50% of the female population died within 24 days, with a few living for 54 days.

FIELD RECOGNITION.—Although the little house fly possesses distinct morphological features which distinguish it from other common Diptera of economic importance, its identity is often mistaken. A few of the more important structural features are presented here to serve as aids for identification of this species.

The chorion of the egg is milky white, approximately 1 mm. in length, and possesses two wing-like processes which extend laterally along its length. The dorsal aspect is characterized by deeply embossed reticulations which are somewhat hexagonal in shape, and a mid-dorsal ridge is visible. The egg is asymmetrical when viewed laterally; the posterior portion being wide and the anterior portion much narrower. The lateral and ventral aspects possess longitudinal ridges which extend discontinuously the length of the egg.

Detailed descriptions of the larva have been made by Greene (1956), Hewitt (1912) and Lewallen (1954). Essentially, the larvae of *F. canicularis* is unlike most immature stages of other common muscoid flies, in that it is not smooth, cylindrical and tapering anteriorly. Instead, it is flattened dorso-ventrally and bears prominent dorsal and lateral fleshy processes (fig. 1). The first and second instars are translucent white, while the third instar is leathery and pale brown. Lewallen (1954) has photographs illustrating the larval instars.

The pupal stage is also somewhat unusual. It has the appearance of a quiescent larva that has ceased feeding, retracted the cephalic region, and becoming somewhat shortened and more robust, with the integument hardened and darkened.

Key characters used by James (1947) and others in separating *F. canicularis* larvae from *F. scalaris* (F.), a closely allied species frequently found in the same environment, are often inadequate for field recognition, since

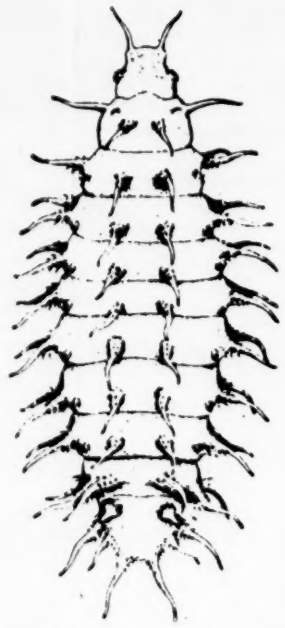

FIG. 1.—Larva of *F. canicularis*. (After Hewitt 1912)

larvae and pupae encountered in the field are usually encrusted with debris, making it difficult to distinguish between the two species by the morphology of their fleshy processes. In these instances, it was found that visual inspection of the ventral abdominal sternites will quickly indicate the identity of most specimens. Larvae and pupae of *F. canicularis* bear a median transverse carina on their ventral abdominal sternites, while *F. scalaris* lacks this fold, its ventral abdominal sclerites being smooth. Also, *F. canicularis* has an anal opening hardened by a raised fold, forming a "V" with the apex anteriorly, while the anal opening of *F. scalaris* is oval in outline and not hardened by a raised fold forming a "V". The latter difference is particularly evident in the pupa.

The adult of *F. canicularis* differs mainly from *Musca domestica* L. by the following characteristics (a) it is slightly smaller in size; (b) the fourth longitudinal wing vein is straight, first posterior cell open, wings held close together when resting, forming a narrow "V"; (c) abdominal coloration dull black, with yellow buff patches on the basal-lateral aspects of the male and only basally on the female; (d) only three brownish-black thoracic stripes, more prominent on the female; (e) flight habits are slow, commonly found congregating in circular or zigzag play flights beneath trees, suspended objects or in the center of rooms and hallways. The combinations of these characters provide an aid to quick field recognition of this species.

OVIPOSITION PREFERENCES.—To determine the attractiveness of manures to this fly, freshly voided samples were placed individually in paper cups and exposed collectively in cages containing gravid females. This procedure was replicated four times and counts of eggs in each medium were made after 4 days. The percentage of the total number of eggs laid were as follows: chicken 63.5, hog 20.5, horse 8.7, cow 4.0 and sheep 3.0. A similar pre-

ference for hen manure was exhibited when the manure samples were exposed for 11-day periods. The high ammonia content associated with chicken droppings may be the attractive factor. These laboratory observations are in accordance with field observations and collections at various locations.

TROPISMS.—The slow flight of this species and its activities in sheltered habitats suggested that it was sensitive to air currents. In the laboratory, caged flies were subjected to air blasts from a high speed rotary impeller. An anemometer in the cage registered the velocity of air flow. Reduction in flight activity was noticed at 9.8 m.p.h. but voluntary directional flight was still normal. At 13.8 m.p.h., directional flight was very unstable and most of the flies clung to the sides of the cage. At 16.6 m.p.h., there was cessation of locomotion and dislodged flies were at the mercy of the air current.

It was also evident in the laboratory that the adults were positively phototropic while the larvae were negatively phototropic.

An adult preference for cool environments was evident in field studies conducted at a poultry farm. During the spring and fall and on overcast days in mid-summer when air temperatures approximated 78° F., flies abounded on the outside walls of buildings. However, when the temperature of wall surfaces exposed to the sun reached 84° F., the flies rested only on the shaded sides and surfaces of buildings where the temperatures were cooler. When air temperature exceeded 86° F., most of the adults did not rest on the outsides of buildings, but retreated to cooler areas, such as hallways, stables, homes, et cetera, where their playflight activities assumed the appearance of black clouds when infestations were heavy.

MANAGEMENT PRACTICES VERSUS INFESTATION.—During the winter of 1957–58, poultry farms across the State were inspected. Of the 29 farms visited, 19 employed the pit system, 7 the open floor, 2 the dropping board and 1 the caged-layer system of droppings management.

The pit system utilizes an enclosure, 1 to 2 feet deep underneath the roosts, where the voided hen manure drops and accumulates. This system is popular with farmers because the bulk of the manure is collected in a given area and minimum contamination of the litter occurs. In addition to the desirability of this system from a sanitation aspect, it allows droppings to be accumulated for long periods, ranging from 1 to 12 months, eliminating the expense of frequent removal.

Estimations of infestation on each farm was based on the presence of larvae and pupal skins and their distribution. Four arbitrary categories were used: none, light, medium and heavy. Of the seven farms which utilized the open floor system, only one was considered as supporting a medium infestation, while on the other six farms traces of an infestation were not detected. Of those farms employing the pit system, 6 had heavy, 4 medium, and 7 light infestations.

In late April and early May of 1958, when *F. canicularis* adults were abundant on farms employing the pit systems, adult flies were scarce on those farms using the open floor system. Since the litter receives all the voided droppings in the open floor system, it must be maintained by tillage, addition of material, adequate ventilation, et cetera, in order that it may serve as a functional absorptive medium. When effectively practiced, this type of management keeps the litter in a dry condition which is not conducive for larval development, and is an effective system for preventing large populations of this fly from developing.

DISTRIBUTION OF LARVAE.—At times, it was difficult to locate overwintering larvae in dropping pits, although their presence was strongly suspected. A study to determine the distribution of larvae in a pit was made in the winter of 1958. Concerning lateral distribution, no larvae were detected in the central area of the pit, although they abounded at the periphery, particularly where vertical construction members provided 90° angles in the pit. Two factors which might explain this distribution are: (1) the apparent preference of gravid females to oviposit on vertical surfaces adjoining the medium, particularly where the medium is wet, and (2) the migrations of maturing larvae from wet to dry portions of the medium, which occurred along the cracks of the supporting boards and in the area of the 90° angles.

Regarding vertical distribution, no larvae were encountered on the surface of the medium, while 17.8%, 48.9% and 33.3% of 287 recovered larvae were at the 1 to 2 inch, 2 to 4 inch and 4 to 6 inch levels, respectively. The temperature of the medium ranged from 40° F. at the surface to a maximum of 58° F. in the 2 to 4-inch layer. However, during the summer months, when the medium was drier, the larvae were on the surface of the manure in the pits.

TEMPERATURE IN DROPPING PIT.—Data collected in a poultry house during November 1957 to August 1958 showed that although the daily air temperature in a pen 5 feet above the manure surface fluctuated as much as 20° F. in a 24-hour period, the manure temperature in the pits at 3 inches below the surface remained fairly constant. The minimum air temperature reached in the upper level of the pen was 38° F. while the minimum manure temperature was only 48° F. In the pits, the larvae were active and capable of slow development at this temperature. In the laboratory, newly hatched larvae emerged as adults 5 months later when kept at 48° F. These data demonstrate the low effective developmental range of this species and explain why *F. canicularis* was the only overwintering muscoid larvae found in abundance.

It was also noted that the mean weekly manure temperature was more closely related to the maximum weekly air temperature than to the minimum weekly air temperature. During late June, July, and early August of 1958, the average manure temperature (85° F.) exceeded the maximum air temperature in the pens (82° F.). At this time, the manure was dry except for the freshly voided droppings on the surface, where the developing larvae were active. The higher temperatures and dryness of the droppings probably suppressed the *Fannia* population during these mid-summer months (fig. 2).

OVERWINTERING STAGES.—All stages of the fly, except the egg, were found during the winter months. The predominant overwintering forms were third larval instars and pupae. The adults occurred only in small numbers in cracks and crevices. When disturbed, they were extremely sluggish and flew with considerable difficulty. However, on days when the air temperature in the pens exceeded

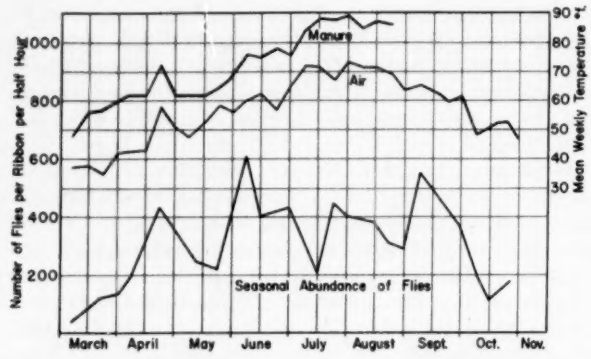

FIG. 2.—Seasonal distribution of *F. canicularis* adults on a poultry farm during 1958, as determined by the numbers caught on fly ribbons exposed for half-hour periods.

45° F., adults were seen flying of their own volition.

SEASONAL DISTRIBUTION.—Activity of the overwintering adults became noticeable in late February, when air temperature in the poultry houses approximated 54° F. On a few farms under observation, emergence from overwintering pupae began before March 10. Phenological data which appeared to coincide with adult emergence were as follows: in 1958, crocuses, which were planted in front of a house exposed to the south appeared on March 1, first showed color on March 10, and were in full bloom by March 15; in 1959 crocuses and snowdrops were in full bloom on March 24, while buds on red maple were greatly swollen and pussy willows had appeared.

Indices of population densities were obtained by suspending fly ribbons in the midst of aerial concentrations of circling flies for half-hour periods. The flies alighted readily on the ribbons where they were captured, and counted with the aid of a hand counter. At least four ribbons were used on a farm on every counting date, and the fly counts for the individual ribbons were averaged to arrive at a *Fannia* index. *Fannia* index counts were made weekly, between noon and 4 p.m.

The seasonal distribution of the little house fly in 1958 is shown in figure 2. The adult population increased steadily during March and early April and reached its first peak during the third week in April. The population densities were greatest during the second week of June and September. The adults did not disappear during the summer months as they sometimes do (Hansens *et al.* 1955, Lewallen 1954) but remained at high levels. At times, adult playflights contained such large numbers of flies and became so dense as to force persons to close their eyes and mouths while walking through them. In late fall, the population declined, although noticeable adult activity persisted until December. The only period during which flies were not captured on ribbons was from January 3, 1957 to February 7, 1957, an interval of approximately 1 month.

There were indications of a correlation between temperature and fly abundance (fig. 2). The maximum number of flies were captured when the mean weekly air and manure temperature approximated 60° to 65° F. and 70° to 80° F., respectively, and mean weekly temperatures above or below these ranges tended to reduce the population. Additional seasonal data are necessary to substantiate these indications.

INVASION OF HOMES.—This study resulted from increasing complaints of annoyance caused by invasion of flies into homes, particularly those near poultry farms. To determine the extent of this occurrence the cooperation of a local complainant was solicited. This residence was old, and in close proximity to a heavily infested poultry farm.

From July 1957 to July 1958, a fly ribbon was hung in place directly over the kitchen table. Each week, the ribbon with its captured flies was removed and replaced. A maximum of 418 flies was collected on a ribbon during the second week of August 1957 (fig. 3). The little house fly was in the kitchen throughout the year, except for a period of approximately 3 weeks during February of 1958. The flies appeared again in early March.

The little house fly was the most abundant species in the house, although the common house fly was numerous at times during the summer. By observation and questioning the occupants, it was evident that the little house fly did not settle readily on exposed human foods.

NATURAL ENEMIES.—During the course of this study six natural enemies of *F. canicularis* and one of *F. scalaris* have been observed and reported previously (Steve 1959).

CONTROL.—Organophosphorous-impregnated fly cords suspended from ceilings have provided extended control of house flies in dairy barns and military dining halls (Kilpatrick 1955, Maier & Mathis 1955, Schoof & Kilpatrick 1957). Similar results were also obtained by Ogden & Kilpatrick (1958) against *F. canicularis* in Utah dairy barns, and by Smith (1958) on California poultry farms. Insecticide-impregnated cords had not been evaluated previously as a control measure against the little house fly in Massachusetts. Such a study was therefore conducted in central Massachusetts in 1959.

PROCEDURES.—Commercially-prepared fly cords (Fly Cord brand) containing 13.79% parathion, 3.54% Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and 82.6% cotton cord, with traces of red vegetable dye and petroleum distillate, were installed on three poultry farms during the summer of 1959. Cords were not installed on a fourth farm, which was intended as an untreated control but became a malathion-treated check, since there were attempts by the farmer to reduce the *Fannia* population with malathion wettable powder sprays.

The cords were installed on the three treated farms according to the manufacturer's recommendations (300 ft. of cord per 1000 sq. ft. of floor space). The cords were placed horizontally, approximately 3 feet apart, and at least 1 foot below the ceiling. A stapling gun was used to attach the cords. In addition to the poultry houses proper, the cords were installed under roofed areas such as garages, grain rooms and machinery sheds, where fly activity was noticeable.

The little house fly was abundant on all of the experimental farms and particularly on farm A (fig. 4). *Fannia* indices of population densities were obtained by suspending fly ribbons in the midst of aerial concentrations of circling flies as previously discussed. At least four ribbons were used on each farm on every counting date to arrive at a *Fannia* index for each farm. *Fannia* index counts

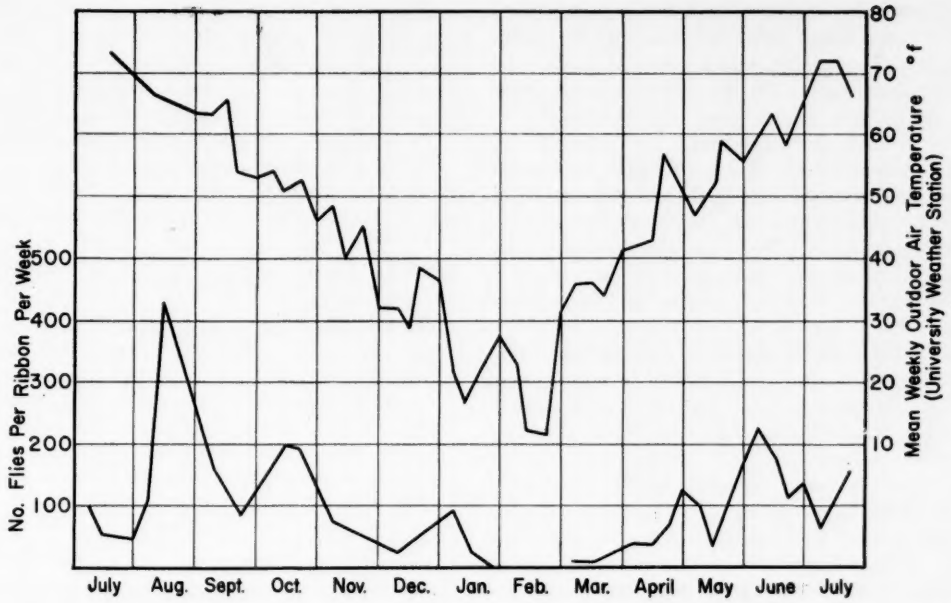

FIG. 3.—Number of adult flies captured when fly ribbons were exposed for weekly periods over the kitchen table in a house located about 100 yards from a poultry farm which showed a heavy infestation of *F. canicularis* in 1957–58.

were made weekly, between 12 noon and 4 p.m., except just prior to and immediately after treatment, when more frequent counts were made.

RESULTS AND DISCUSSION.—The effectiveness of impregnated fly cords against the little house fly is illustrated in figure 4. A decline in the *Fannia* population was always noticeable on the day following cord installation, and on farms A and B, satisfactory control levels were reached within 4 days. A *Fannia* index of 25 or less flies per ribbon per half-hour period in favored areas represented a low population and was considered a satisfactory control level.

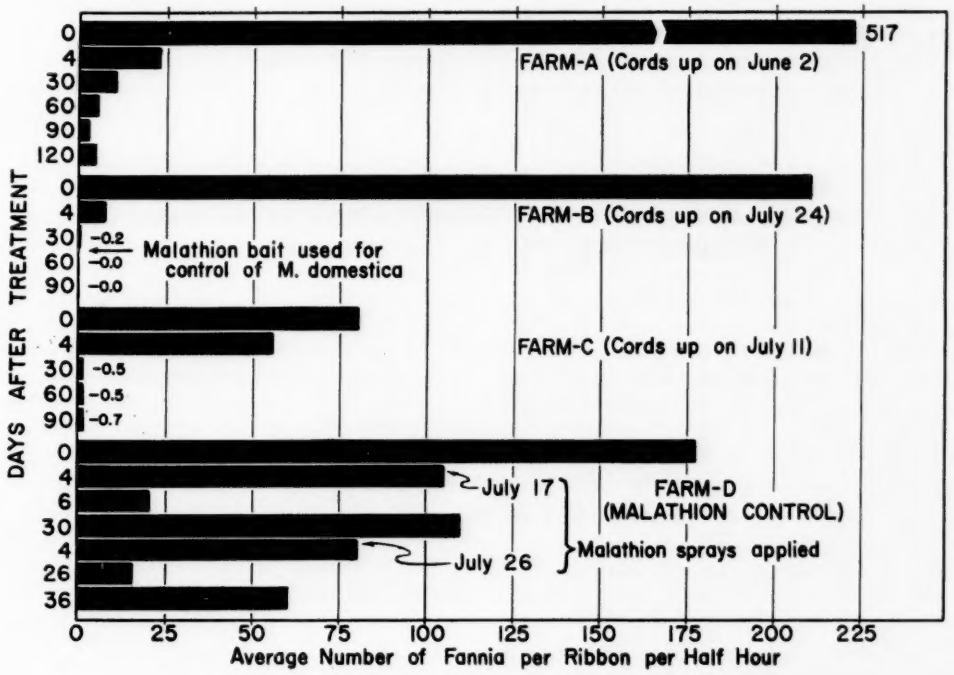

FIG. 4.—Reduction of *F. canicularis* adults on three poultry farms treated with impregnated cords (parathion at 13.7% and Diazinon at 3.5%) 1959.

The reduction of flies on farm C was slower and the control level was not reached until the 12th day following cord installation. This delay was attributed mainly to these facts: (1) about half of the cords in a barn, separate from the main poultry house, were dislodged and had fallen to the ground, resulting from the attempts of chickens to roost upon them and, (2) no cord was installed in a small tool shed with a low ceiling, since it would have interfered with use of the area. Although the ribbon counts in the main poultry house at this location indicated a rapid reduction, the high counts in the barn and tool shed held up the *Fannia* index for this farm for a longer period.

In contrast to the malathion-treated control farm, population buildups above the control level did not occur after cord installation on any of the three cord-treated farms during the rest of the summer (up to 120 days), at the end of which observations were discontinued (fig. 4). The malathion sprays on the control farm were applied by the owner, and although temporary control was obtained, subsequent population buildups occurred after both applications. On treated farms B and C, adults of the little house fly remained scarce throughout the season after the cords were installed.

Prior to the time the cords were installed, it had been observed that the use of malathion sugar baits caused reductions in populations of *M. domestica* but not of *F. canicularis*. The population of *M. domestica* was reduced on each of the three farms after cord installation, but house fly populations increased during mid-August at all locations. At this time, the use of a malathion bait by the owner of farm B was effective against *M. domestica*. Thus, although the treated cords proved extremely effective against *F. canicularis* for the entire season, additional control measures were considered necessary to suppress *M. domestica* at this location.

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Analog-Synergism of Several Carbamate Insecticides[1]

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ABSTRACT

Combinations of two carbamate insecticides applied jointly were found to increase mortality beyond the expected additive effect on two of the three insect species tested. Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate) was found to synergize the more potent *in vitro* cholinesterase inhibitors Sevin® (1-naphthyl methylcarbamate) and Pyrolan® (5-(3-methyl-1-phenylpyrazolyl) dimethylcarbamate) on German cockroaches (*Blattella germanica* (L.)) and house flies (*Musca domestica* L.). It was suggested that those two species possess a detoxifying enzyme "carbamate esterase" which destroys the carbamate insecticides. Partial inhibition of this enzyme by Dimetilan was suggested as an explanation for its synergistic action with other carbamate insecticides that are broken down easily by the detoxifying enzyme. The relative toxicity of the tested carbamates to the large milkweed bug (*Oncopeltus fasciatus* (Dall.)) was different from flies and cockroaches and there was no apparent synergism.

Eldefrawi *et al.* (1960) have shown that Dimetilan is 20 times more toxic to house flies than Pyrolan or Sevin, although it is a much weaker inhibitor of fly-head cholinesterase *in vitro*. Roan & Maeda (1954) had previously observed a similar effect on three species of fruit flies. The simplest explanation of this effect would be the presence of a "carbamate-esterase" that can hydrolyze Sevin and Pyrolan quickly but Dimetilan only slowly. This suggested to us the possibility that Dimetilan might form a stable complex with the "carbamate-esterase" and so block the enzymatic hydrolysis of Sevin and Pyrolan. If

[1] This work was supported by Research Grant E-1081 (c) from the National Institutes of Health, U. S. Public Health Service. Synergism between carbamate insecticides was independently discovered in our laboratory by S. Hassan, in testing dilute aqueous solutions on mosquito larvae; this effect differs in some ways from those reported here, and will be published in a separate paper.

Accepted for publication May 4, 1960.

so. Dimetilan would be a synergist for its potentially much more cholinesterase-inhibiting structural analogs. Analog-synergism is a well-known phenomenon in pharmacology, *e.g.*, the action of epinephrine is strongly potentiated by its analog, p-hydroxybenzedrine, which has a similar but weaker and longer-lasting action (Issekutz 1939). More recently the "potentiation" between malathion and EPN in the rat and the dog has aroused interest (Frawley *et al.* 1957).

Moorefield (1958) showed that on house flies the pyrethrin synergist, piperonyl butoxide, at dosages of about 0.5 μg./fly, lowers the LD_{50} of Sevin and Pyrolan from about 2 to 0.1 μg./fly. Eldefrawi *et al.* (1959, 1960) showed that the stronger synergist, sesamex (Sesoxane) can lower the LD_{50} of Sevin and Pyrolan to 0.05 μg./fly for "susceptible" house flies, and that DDT-resistant and parathion-resistant flies (which show cross-resistance to carbamates) are not highly resistant to carbamate-synergist combinations.

MATERIALS AND METHODS.—The four carbamates used by Eldefrawi *et al.* (1960) were used in this study: Sevin® (1-naphthyl methylcarbamate), Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate), Pyrolan® (5-(3-methyl-1-phenylpyrazolyl) dimethylcarbamate), and Isolan® (5-(1-isopropyl-3-methylpyrazolyl) dimethylcarbamate). Sesamex (Sesoxane) was used as a synergist in some tests.

House flies (*Musca domestica* L.) were the "susceptible" strain maintained in our laboratory for several years without intentional exposure to insecticides. It must be noted that the topical LD_{50} of DDT to these flies is nearly 10 times higher than that of house fly strains tested before DDT-resistance became wide-spread. The insecticides were applied to the notum of 2- to 4-day-old female flies (weighing about 20 mg.), in acetone solution, using 1- or 2-microliter self-filling capillary pipets. When two substances were tested jointly, they were usually in one solution; in some tests, similar results were obtained when two different solutions were applied separately to the same site, within 30 seconds. Twenty-five female flies were used per test.

The large milkweed bugs (*Oncopeltus fasciatus* (Dall.)) were last-instar nymphs weighing 20 to 30 mg., given to us by Dr. J. J. Menn of the Agricultural Research Laboratories, Stauffer Chemical Company. Insecticide solutions in acetone were applied to the dorsal region of the thorax. Ten bugs were used per test.

German cockroaches (*Blattella germanica* (L.)) were reared in our laboratory. Only males, weighing about 50 mg., were used. Insecticide solutions in acetone were applied to the ventral thoracic region. Ten cockroaches were used per test.

All mortality counts were made 48 hours after application.

RESULTS AND DISCUSSION.—*Analog-Synergism on the House Fly.*—The data are given in table 1. Useful synergism is shown when the observed mortality is higher than the "additive per cent mortality." Toxicities in this discussion will all be expressed as μg./gram of body weight, to facilitate comparison between insect species; in the tables, dosages are given in μg./insect because this is the convenient unit for experimental work.

Pyrolan and Sevin are relatively nontoxic to the house fly (LD_{50}:100 μg./gm.) but are strongly synergized by sesamex, which can lower the LD_{50} to about 5 μg./gm. Isolan is more toxic (LD_{50}:25 μg./gm.), and Dimetilan is the most toxic analog (LD_{50}:5 μg./gm.), but these analogs are more weakly synergized by sesamex, the LD_{50} being lowered to about 2 to 5 μg./gm. Roan & Maeda (1954) found that three species of fruit flies were even more susceptible to carbamates, the LD_{50} of Pyrolan being 1 to 2 μg./gm. and of Dimetilan 0.5 to 1 μg./gm. (without any synergist). It seems that the ultimate toxicity of the four carbamates to flies is not very different (lying near 2 μg./gm.), but that Sevin and Pyrolan cannot attain this toxicity unless the hypothetical "carbamate esterase" is blocked by a suitable synergist. Dimetilan and Isolan are presumably destroyed more slowly by the "carbamate esterase," and so are not strongly synergized by sesamex.

The data on analog-synergism show, as expected, that combinations of Dimetilan-Sevin and Dimetilan-Pyrolan give more than additive kill on house flies (table 1). However, the synergism is very weak unless Dimetilan is used at a dosage high enough to give considerable mortality if used alone. Thus, 0.02 μg. of Dimetilan (near the LD_0) causes little or no increase in the kill caused by 0.5 μg. of Sevin. At 0.05 μg. of Dimetilan (near the LD_{20}), addition of 0.2 μg. of Sevin gives a mortality increment equal to that which would be caused by the addition of 0.05 μg. of

Table 1.—Toxicity of carbamate insecticides, singly and in pairs, by topical application to the notum of "susceptible" female house flies.

Dosage in μg./Fly of					Maximal Additive Kill (%)	Observed[a] Kill at 48 Hours (%)
Dimetilan	Isolan	Sevin	Pyrolan	Sesamex		
0.05	—	—	—	—	—	15–20
0.10	—	—	—	—	—	30–50
0.50	—	—	—	—	—	90–95
—	0.10	—	—	—	—	5–10
—	0.40	—	—	—	—	25–35
—	0.50	—	—	—	—	50
—	—	1.0	—	—	—	5–20
—	—	2.0	—	—	—	45–55
—	—	—	1.0	—	—	10–20
—	—	—	2.0	—	—	30–60
0.02	—	0.5	—	—	20	15
0.05	—	0.05	—	—	20	60
0.05	—	0.20	—	—	20	35–45
0.10	—	0.10	—	—	50	75–95
0.025	—	0.025	—	0.025	10	0
0.025	—	0.025	—	0.20	50	90
0.05	—	0.05	—	0.05	60	65
0.05	—	0.05	—	0.2	60	100
0.05	—	0.05	—	1.0	60	65
0.05	—	—	0.1	—	0	5
0.05	—	—	0.2	—	25	45
0.10	—	—	0.10	—	50	72–95
0.10	0.10	—	—	—	60	90–95
—	0.05	0.1	—	—	10	20
—	0.10	0.5	—	—	15	25
—	0.40	0.1	—	—	35	40
	0.40	0.2				20
	0.40	0.4				28
—	0.05	—	0.10	—	10	5
—	0.10	—	0.50	—	15	10
—	0.40	—	0.10	—	35	60
	0.4		0.2			8
	0.4		0.4			8
—	—	1.0	0.10	—	20	60
—	—	2.0	0.10	—	55	65
—	—	1.0	1.0	—	40	85
—	—	0.10	1.0	—	20	25
—	—	0.10	2.0	—	60	100

[a] Mortality varied somewhat when an experiment was repeated with a new group of flies of the same "susceptible" strain, so the lowest and highest value observed are both given. For calculation of additive mortalities, only the highest observed values are used.

Dimetilan, *i.e.*, Sevin is effectively one-fourth as toxic as Dimetilan (instead of 1/20th as toxic). At a dosage of 0.1 μg. of Dimetilan (near the LD_{50}), addition of 0.1 μg. of Sevin gives a mortality increment equal to that which would be caused by addition of 0.4 μg. of Dimetilan, *i.e.*, Sevin is effectively four times *more* toxic than Dimetilan, and the combination is a 2-fold stronger insecticide than Dimetilan itself. This is true only at the LD_{95}, because the analog-synergized mortality curve is extremely steep. At the LD_{50}, the 1:1 mixture of Dimetilan-Sevin is no more toxic than pure Dimetilan, and at the LD_{10} it is less toxic.

Combinations of Isolan-Sevin, Isolan-Pyrolan on house flies showed no synergism but it actually suggested antagonism; work in progress in our laboratories with other carbamate combinations on house flies strongly suggests antagonism.

The conditions for efficient analog-synergism seem to be the combination of an LD_{50} dose of a moderately toxic, slowly detoxified analog (the "synergor") with an equal or somewhat lower dose of a potentially much more toxic but rapidly detoxified analog (the "synergee"). We propose that the term "synergist" be restricted to compounds that are not themselves toxic at synergistically effective dosage, *e.g.*, piperonyl butoxide used as a pyrethrin or Sevin synergist, or Dimite® (4,4′-dichloro-*alpha*-methylbenzhydrol), Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl) benzhydrol), or other DDT-analogs used as synergists for DDT on DDT-resistant house flies.

The high toxicity of Sevin and Pyrolan revealed both by analog-synergism and sesamex-synergism, agrees with the data of Eldefrawi *et al.* (1960) on the relative *in vitro* anticholinesterase activity on crude house fly head esterase. The I_{50} values (all X 10^{-7} M) are 6.6 for Pyrolan, 30 for Sevin, 50 for Isolan, and 160 for Dimetilan. These values predict (and the analog-synergism roughly confirms) that Sevin and Pyrolan should be some 5 to 10 times more toxic than Dimetilan. However, it must be noted that nonanalog synergists like sesamex cannot, at any dosage, lower the LD_{50} of Sevin and Pyrolan below that of Dimetilan. Unless we assume that sesamex can never cause more than partial inhibition of "carbamate esterase," we must conclude that Dimetilan does more than block only "carbamate esterase." In fact, at its synergistically efficient (toxic) dosage, Dimetilan is also inhibiting true cholinesterase and causing prolonged knockdown of all the flies. As a control, flies were treated with approximately LD_{50} doses of Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) (0.2 μg./fly), malathion (0.2 μg./fly), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate 60% technical) (0.02 μg./fly), and phorate (0.08 μg./fly), with and without the addition of 0.2 μg. of Sevin. The Sevin caused a slight decrease in mortality; this kind of antagonism also occurs *in vitro* (Metcalf & March 1950), and is usually explained as a protection of cholinesterase by carbamate bound to the enzymatic site from the less reversible effect of the organic phosphate. It is therefore impossible to evaluate the effect of direct cholinesterase inhibition in Dimetilan synergism by analogy with organic phosphates.

As a control for simulation of the fast, long-lasting knockdown caused by Dimetilan, flies were treated with an LD_{50} dose of allethrin (0.5 μg./fly), with and without the addition of 0.2 μg. of Sevin. The Sevin caused only a slight increase in mortality. This proves that mere knockdown-paralysis is not the important factor in Dimetilan synergism.

As a control for evaluation of the possible inhibition of "carbamate esterase" by Dimetilan, 1:1 Dimetilan-Sevin mixtures were tested with added sesamex. The data in table 1 show that 0.05 μg./fly of sesamex has no effect, although 0.2 μg./fly does synergize Dimetilan-Sevin. This suggests that Dimetilan may not completely protect Sevin from hydrolysis by "carbamate esterase." Eldefrawi *et al.* (1959) have shown that as little as 0.02 μg./fly of sesamex increases mortality from LD_{50} doses of either Dimetilan or Sevin. The best inference seems to be that sesamex can protect Dimetilan, improve the protection of Sevin, and prolong the synergistic action of Dimetilan on Sevin on nerve cholinesterase.

Mengle & Casida (1958) found that the cholinesterase inhibition in the heads of surviving flies treated with an LD_{50} dose of Isolan was as intense and much more long-lasting than that caused by an LD_{50} dose of Sevin, although Sevin is a stronger *in vitro* anticholinesterase and must be applied in a 5-fold larger dose. This is consistent with the hypothesis that the toxicity of carbamates to house flies is determined by their rate of hydrolysis by "carbamate esterase" rather than by their I_{50} value on cholinesterase *in vitro*. Kolbezen *et al.* (1954) implied that there was good correlation between the I_{50} and LD_{50} values of a series of substituted phenyl N-methylcarbamates tested on house flies, but an examination of their data shows that this is true only insofar as many of the analogs tested were both nontoxic and extremely weak anticholinesterases. For analogs that were relatively toxic to flies or relatively strong anticholinesterases, there were many examples of noncorrelation; *e.g.*, the I_{50} (X 10^{-7} M) of the *m*-methyl analog is 80 and that of the *m*-tert-butyl analog is 4, but both are equally toxic (LD_{50} about 50 μg./gm. or 1 μg./fly). Similarly, the most toxic analog to flies is the 5-methyl-2-isopropyl (LD_{50} about 30 μg./gm.); its isomer, the 2-methyl-5-isopropyl analog, is nontoxic but has the same I_{50} (20 X 10^{-7} M). The toxicity of these analogs to *Heliothrips haemorrhoidalis* (Bouché), however, did show good positive correlation with their I_{50} values on house fly head cholinesterase; the point is that *in house flies* there are factors that tend to make the correlation *negative*.

Metcalf and his coworkers (1950, 1955, 1956) have shown that the house fly brain has an extraordinarily high level of true cholinesterase, and unusually low levels of other esterases, when compared with the brains of other (nondipteran) insects. The work of Goldstein (1951) on the kinetics of cholinesterase inhibition led him to conclude that carbamates, unlike other cholinesterase inhibitors, "are better considered to be competitive substrates with exceptionally low turnover rates." Later work proved that true cholinesterase does catalyze the slow hydrolysis of carbamates (Goldstein & Hamlisch 1952), and that physostigmine (I_{50} about X 10^{-8} M), although a stronger inhibitor than prostigmine (I_{50} about 5 X 10^{-8} M) is hydrolyzed over four times faster by the enzyme. This suggests that there may be a negative correlation between the I_{50} and the hydrolysis rate by cholinesterase (and possibly also by other esterases). We can calculate the

order of magnitude of the hydrolysis of carbamates in the house fly brain, since Goldstein & Hamlisch (1952) found that the hydrolysis rate is about one-millionth that of acetylcholine, and Metcalf & March (1950) found that one fly brain hydrolyses about 4 micromoles of acetylcholine per hour; the maximum hydrolysis rate for carbamates like Pyrolan and Sevin (which are strong anticholinesterases like physostigmine) is then of the order of 0.001 μg./hour, while for the weaker anticholinesterases like Dimetilan it may be 0.0001 μg./hour or less. The data of Mengle & Casida (1958) suggest that the greater part of a 1 to 2 μg. dose of Sevin can be destroyed in a house fly in 5 to 6 hours, *i.e.*, a total hydrolysis rate of about 0.4 μg./hour, or 400 times that which the brain cholinesterase alone could give; for Isolan, the similarly estimated total hydrolysis rate is less than 0.1 μg./hour. This suggests that the greater part of the hydrolysis of carbamates must be effected, not by brain cholinesterase, but by "carbamate esterase," and that the latter enzyme, like true cholinesterase, is usually more active in hydrolyzing carbamates that are strong anticholinesterases. We can now interpret some of the data of Kolbezen *et al.* (1954), on carbamates that have nearly the same I_{50} but strikingly different LD_{50} on flies. There are three examples in which addition of an *o*-methyl group does not affect the I_{50}, but greatly lowers the toxicity to flies; these are the *o*-methyl analog, the 2-methyl-5-isopropyl analog, and the 2,3,5-trimethyl analog. This "*o*-methyl effect" must be caused by more rapid hydrolysis of such a structure by "carbamate esterase." It is possible that such a structure is also hydrolyzed more rapidly by cholinesterase, but the absolute rate of hydrolysis is so much lower that it does not affect I_{50} or LD_{50}.

Weak Synergism of Carbamates on the Large Milkweed Bug.—The data in table 2 show that carbamates are not highly toxic to *Oncopeltus* nymphs; the most effective one, Sevin, has an LD_{50} of about 20 μg./gram. In these insects, however, marked symptoms of intoxication may persist for 3 days before the insects recover or die, and it is clear that the low toxicity merely reflects the fact that they are hard to kill, not that their nervous system is unaffected. The order of toxicity: Sevin > Isolan > Dimetilan shows good correlation with anticholinesterase values on house fly brain enzyme. This agrees with the results of Kolbezen *et al.* (1954) on carbamate toxicities to an hemipteroid of the order Thysanoptera, *Heliothrips haemorrhoidalis*. These facts suggest that hemipteroids may have negligible "carbamate esterase" activity. Metcalf *et al.* (1955) showed that the cholinesterase activity (per milligram) in the head of the squash bug, *Anasa tristis* (DeG.), is only 3% of that in the house fly; if the content of other esterases is similarly low, toxicity should be primarily determined by anticholinesterase activity. This view is supported by the extremely weak synergistic action of sesamex, which can have little effect on toxicity if there is little or no detoxicating enzyme for it to inhibit.

There is one exception, the unexpectedly low toxicity of Pyrolan; even at high dosage it does not cause the striking knockdown and intoxication caused by Sevin and Isolan. Among the strong anticholinesterase phenyl N-methylcarbamates studied by Kolbezen *et al.* (1954) on thrips, there were two nontoxic exceptions (the *m*-methyl analog and the *o*-chloro analog) but these could be accounted for by their relatively rapid nonenzymatic hydrolysis in dilute alkaline solution. Unpublished observations by R. P. Miskus in our laboratory indicate that Pyrolan is easily hydrolyzed by dilute alkali (where Dimetilan is very slowly hydrolyzed); Pyrolan is also a bulkier and a less water-soluble molecule than the other carbamates, and may penetrate and diffuse rather slowly in the milkweed bug; even in house flies its knockdown action is rather slow.

Table 2.—Toxicity of carbamate insecticides, singly and in pairs, by topical application to the dorsum of last-instar milkweed bugs.

Dosage in μg./Bug of					Maximal Additive Kill (%)	Observed Kill at 48 Hours (%)
Sevin	Isolan	Dimetilan	Pyrolan	Sesamex		
0.125	—	—	—	—	—	10
0.25	—	—	—	—	—	20
0.5	—	—	—	—	—	40
1.0	—	—	—	—	—	100
—	0.5	—	—	—	—	20
—	1.0	—	—	—	—	100
—	—	1.0	—	—	—	0
—	—	10.0	—	—	—	20
—	—	—	1.0	—	—	10
—	—	—	10.0	—	—	10
0.125	—	—	—	1	10	30
—	—	—	5.0	5	10	20
0.25	0.25	—	—	—	30	90
0.125	—	1.0	—	—	10	30
—	—	5.0	5.0	—	20	10

The difficulties of interpreting toxicity in terms of *in vitro* enzyme actions are much greater than was anticipated in early studies of cholinesterase inhibition by insecticidal phosphate and carbamate esters. Like the phosphates, carbamates can display striking differences; *e.g.*, Pulver & Domenjoz (1951) showed that Pyrolan and physostigmine are both powerful inhibitors of human plasma pseudocholinesterase, and also of human plasma novocaine-esterase; Pyrolan, however, is only one-twentieth as active as physostigmine on human red cell cholinesterase, while it is over 1000 times *more* active than physostigmine on the "parpanit-esterase" of rabbit plasma. Similarly, Metcalf & March (1950) showed that fly head esterase is 100 times more sensitive than bee head esterase to the synthetic carbamate Nu-683, the dimethylcarbamate of (2-hydroxy-5-phenylbenzyl) dimethyl ammonium chloride, but only four times more sensitive to physostigmine. It is therefore possible that the low toxicity of Pyrolan to some insects is owing to a qualitative difference in nerve cholinesterase.

Table 2 shows that analog-synergism is possible, but the only promising combination is a 1:1 Isolan-Sevin, and this is less than twice as toxic as Sevin or Isolan alone.

Analog-Synergism on the German Cockroach.—The data in table 3 show that carbamates are not highly toxic to male *Blattella germanica*; the most effective one, Isolan, has an LD_{50} of about 25 μg./gram. Like large milkweed bugs, cockroaches are hard to kill, and may recover after 2 days of severe intoxication and knockdown. Except for their greater toughness, however, cockroaches react to carbamates rather like house flies. Sevin and Pyrolan are nontoxic (LD_{50} 400 μg./gram) but addition of sesoxane makes them moderately toxic (LD_{50} about 100 μg./gram). Doses of Isolan or Dimetilan in the LD_{20} to LD_{50} range

Table 3.—Toxicity of carbamate insecticides, singly and in pairs, by topical application to ventral thorax of male German cockroaches.

Dosage in μg./Cockroach of					Maximal Additive Kill (%)	Observed Kill at 48 Hours (%)
Isolan	Dimetilan	Sevin	Pyrolan	Sesamex		
1.0	—	—	—	—	—	20
2.0	—	—	—	—	—	60
—	2.5	—	—	—	—	10
—	5.0	—	—	—	—	60
—	—	7.5	—	—	—	10
—	—	15.0	—	—	—	40
—	—	—	15.0	—	—	0
1.0	—	—	—	10	20	50
—	2.5	—	—	10	10	30
—	—	1.0	—	5	0	10
—	—	5	—	5	5	90
—	—	—	1	5	0	0
—	—	—	10	10	0	90
1	—	1	—	—	20	0
1	—	7.5	—	—	30	100
2	—	1	—	—	60	70
2	—	2	—	—	60	100
1	—	—	7.5	—	20	100
2	—	—	2	—	60	100
—	2.5	7.5	—	—	20	90
—	5	—	15	—	60	50
1	1	1	—	—	20	30
1	—	1	—	1	20	100

give strong analog-synergism with either Sevin or Pyrolan, and 1:1 Isolan-Sevin can be further synergized by sesoxane.

The fact that Isolan is more toxic than Dimetilan suggests that the cockroach may have less "carbamate esterase" than the house fly, or different substrate specificity. An LD_{50} dose of Isolan may not be largely destroyed until 48 hours or longer after application (a total hydrolysis rate of the order of 0.04 μg./hour, which is less than one-fourth the activity per mg. body weight, compared with the house fly); therefore, the higher anticholinesterase activity of Isolan outweighs the presumably greater stability of Dimetilan. Sevin and Pyrolan, however, are not stable enough to maintain a sufficiently prolonged cholinesterase inhibition to cause death. It is possible that a large fraction (10% to 20%) of the carbamate hydrolysis takes place within the nervous system of the cockroach, where it would be most effective. Metcalf *et al.* (1955, 1956) showed that the head and nerve cord of the American cockroach, *Periplaneta americana* (L.), contain significant amounts of "aromatic esterase," which is not inhibited by (and can probably hydrolyze) both para-oxon and carbamates like physostigmine.

The Relation of "Carbamate Esterase" to Other Insect Esterases.—Metcalf *et al.* (1955, 1956) showed that there are at least three distinct esterases in house flies, honeybees, and cockroaches. All can hydrolyze a variety of substrates, including acetylcholine and phenyl acetate. The true cholinesterase (ChE) is most active on acetyl and propionyl esters, and shows a remarkable specificity for the acetyl ester of 3,3-dimethylbutan-1-ol. It is inhibited by organophosphate and carbamate insecticides, but can slowly recover from the inhibition; the recovery is probably owing to extremely slow hydrolysis of the inhibitor by the enzyme itself. The aliesterase (AliE) is less specific, and actively hydrolyses esters of most aliphatic acids (from C_2 to C_6), showing considerable specificity for relatively water-insoluble esters such as ethyl valerate or amyl butyrate. It is inhibited by organophosphate insecticides, but not by carbamates (at least at concentrations that give near 100% inhibition of ChE); it may be able to hydrolyze carbamates more rapidly than does ChE. The aromatic esterase (ArE) is most active on esters of a great variety of phenols with the lower aliphatic acids (acetates, propionates, butyrates), but also hydrolyzes most of the substrates attacked by AliE and ChE. It also hydrolyzes esters of aromatic acids (such as methyl benzoate) and phosphate esters such as para-oxon, but at a rate 10% to 20% of the rate for phenyl acetate. It is by far the least specific of the insect esterases. It is only slightly inhibited (in its action on p-nitrophenyl acetate) by 0.01–0.001 M para-oxon or parathion, and the inhibition is of the competitive-substrate type. Metcalf *et al.* (1956) showed that its action on phenyl acetate is not inhibited by 0.001 M of *m*-tert-butylphenyl N-methyl carbamate, an insecticide which Kolbezen *et al.* (1954) showed to be an extremely powerful anticholinesterase (I_{50} on fly head esterase about 4×10^{-7} M). This suggests that the enzyme has such a low affinity for strong-anticholinesterase carbamates that they cannot affect the hydrolysis of substrates having a high affinity; nevertheless, it may hydrolyze carbamates at a very slow rate, perhaps less than one-hundredth of that of phenyl acetate. The data of Metcalf *et al.* (1955, 1956) indicate that the aromatic esterase in the head of one American cockroach can hydrolyze phenyl acetate at the rate of about 1 micromole/hour, while the gut and other tissues may contain enough esterase to hydrolyze more than 10 micromoles/hour. This is a rate of about 0.01 micromoles/mg. of whole insect/hour; carbamates could be hydrolyzed at a rate of 0.001 without showing any obvious inhibition effects on phenyl esterase. This would represent a destruction rate for Sevin of about 0.02 μg./mg. insect/hour; in a female house fly, the rate would be about 0.4 μg./hour. This rate is exactly that estimated from the data of Mengle & Casida (1958), in the previous discussion on carbamate action on house flies. "Carbamate esterase" activity may therefore be only one of the very weak enzyme-substrate interactions of insect aromatic esterase. This hypothesis requires that Dimetilan be an effective inhibitor even of the hydrolysis of phenyl acetate by aromatic esterase, although strong-anticholinesterase carbamates are not.

Certain Practical Considerations Concerning Analog-Synergism.—It may be argued that the testing of all combinations of two toxic analogs will be impractical, since the number of combinations of N analogs taken two at a time is $N!/2(N-2)!$. Even with only 10 analogs, the number of combination tests is 45; with 100 analogs, 4950 tests would be required. However, one of the principles derivable from our work is that promising combinations can often be inferred by inspection of existing routine screening data. If analog A (*e.g.*, Dimetilan) is the most toxic of a large number of analogs to one or more insect species (*e.g.*, house flies), and analog B (*e.g.*, Sevin) is the most toxic to other insect species (*e.g.*, milkweed bugs), a 1:1 combination of A and B, in a single test on each of two species at

two times the LD_{50} dosage of the more toxic analog, may give higher mortality. In this way, the number of analogs worth testing in combinations may be reduced to as few as 3 or 4, and the number of tests to 3 to 6. Analog-synergistic combinations will therefore be rather easy to find.

It may not be practical to develop most such combinations, however, if the high cost of manufacturing and testing two analogs on a large scale is not compensated by the relatively small gain in toxicity. Perhaps more important than the increased toxicity will be the wider range of insect species controllable by combinations of two analogs; *e.g.*, Sevin alone would give poor control of many Diptera, and Dimetilan alone poor control of many Hemiptera, but the combination would act on both groups.

The problem of "potentiation" in chronic toxicity to mammals, as in the well-known example of malathion and EPN (Frawley *et al.* 1957, Williams *et al.* 1958) is unlikely to occur with carbamates. Even among the phosphates, which (unlike carbamates) cause nearly irreversible (and so cumulative) inhibition of mammalian red cell and plasma cholinesterase, potentiation is a rare phenomenon, and is serious only between two exceptionally "safe" phosphates: the residue tolerance for most phosphates is 1 p.p.m. or less, while that for EPN is 3 p.p.m. and for malathion 8 p.p.m. Carbamate analog synergism acts at levels causing acute toxicity, and an analog-synergized combination may well be more hazardous to handle than either analog alone, but at trace residue levels in food there can be no interactions or cumulative effects.

In the field of insecticide resistance, it would be unduly optimistic to expect, as did Moorefield (1958), that "synergistic carbamate combinations . . . could provide . . . possible suppression of insecticide resistance," or as did Eldefrawi *et al.* (1959), that "effective synergistic action . . . with Sevin gives hope for control of DDT- and phosphate-resistant insects." Synergized insecticides are not resistance-proof. Years of research on synergists for DDT proved that synergized DDT cannot be as toxic to DDT-resistant strains as DDT alone to susceptible strains of house flies; and DDT-resistant strains can rapidly acquire resistance to DDT-synergist combinations, simply by increasing the level of DDT-dehydrochlorinase (Moorefield & Kearns 1955). Eldefrawi *et al.* (1959) have shown that parathion-resistant house flies, never exposed to Sevin or any other carbamate, have considerable cross-resistance even to Sevin-sesamex combinations. Analog-synergism does have one possible advantage, however, in that both analogs are toxic and may be mutually synergistic, if they are destroyed by different detoxication mechanisms. Each analog may then block the mechanism that acts on the other, and a combination of two analogs might be toxic to a strain resistant to both analogs. This would present a more difficult problem to the resistant individuals in an insect population, and might delay the development of high resistance and so prolong the useful life of new insecticides.

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Bee Repellent Combined with Dieldrin or Sevin in Bee Poisoning Tests in Alfalfa[1]

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ABSTRACT

A bee repellent, R-874 (2-hydroxyethyl-n-octyl sulfide), provided good protection against bee poisoning when mixed with sprays of either dieldrin or Sevin®(1-napthyl methylcarbamate) in tests on blooming alfalfa. Honey bees (*Apis mellifera* L.) and bumblebees (*Bombus* spp.) in the fields were repelled effectively for 6 to 8 days when the average maximum temperature for the week following application was 71° F.; for 3 to 4 days when the average maximum temperature was 87° F.

Investigations were conducted in the Pullman area of Washington during 1959 to evaluate a new bee repellent, R-874, developed largely through the efforts of G. A. Bieberdorf in Oklahoma. Some 700 materials had been tested as repellents against various arthropods at the Oklahoma Experiment Station prior to 1952. Bieberdorf (1958) tested the most promising of these for repellency to honey bees and found 2-hydroxyethyl-tert-dodecyl sulfide to be the best. Phillips Petroleum Company chemists synthesized a large number of closely related compounds that were subsequently tested. Compound R-874) (2-hydroxyethyl-n-octyl sulfide) showed the greatest repellent properties of the materials investigated (Bieberdorf & Howell 1959).

MATERIALS AND METHODS.—Eight alfalfa plots, about 1 acre each and surrounded by wheat, other small grains, or alfalfa stubble were established in the Pullman area in 1959. Each of these plots was 2 to 16 aerial miles from any of the others and several miles from any large quantity of honey bee forage plants. Two of these plots were treated with 1.5 lb. actual Sevin® (1-naphthyl methylcarbamate) per acre; two, with 1.5 lb. actual Sevin plus 10 fl. oz. R-874 (repellent) plus 1 fl. oz. Atlox 1045 A (Atlas Powder Co.) emulsifier per acre; two with 0.5 lb. actual dieldrin per acre; and two, with 0.5 lb. actual dieldrin plus 10 fl. oz. R-874 per acre. Two additional alfalfa plots were used as untreated checks. Treatments were made with a truck-mounted horizontal boom sprayer using 125 p.s.i. liquid pressure and applying 15 gallons per acre. Treatments were applied just before maximum bloom for the field in each case (about 150 blooms per square yard where a "bloom" is defined as a raceme with one or more open florets).

Two hives of bees were placed near the center of each plot 1 to 5 days before treatment. Each hive was fitted with a combination dead-bee-pollen trap and a dead-bee pan. The dead-bee-pollen traps were patterned after the one described by Nye (1959). These were modified so that a screen and a drawer at the rear could be used to

[1] Scientific Paper 1941. Washington Agricultural Experiment Stations. Work conducted under Project No. 1419, partially supported through grants from California Spray-Chemical Corp., Shell Chemical Corp., and Stauffer Chemical Co. Accepted for publication May 9, 1960.

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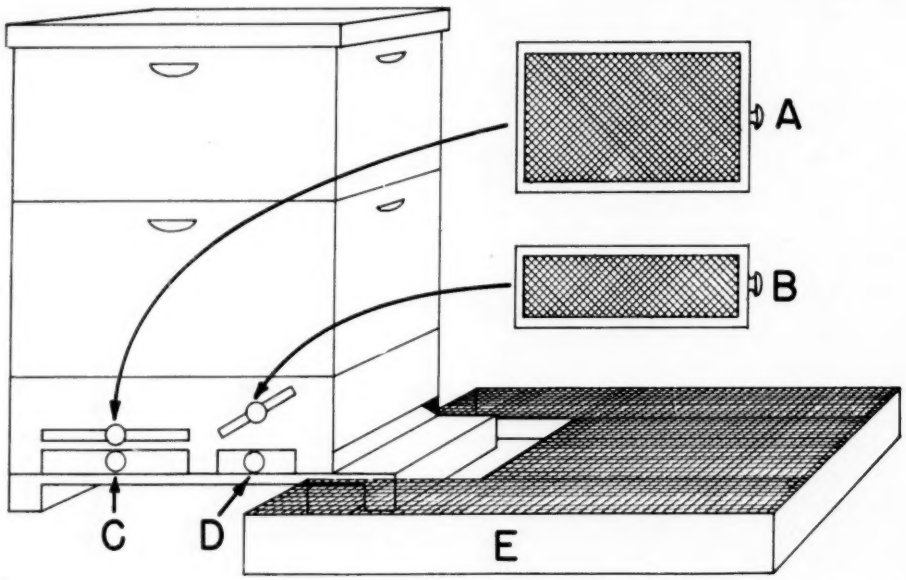

FIG. 1.—Combination dead-bee-pollen trap and dead-bee pan as attached to beehive. A: 2- mesh dead bee screen allows bees dying inside hive to drop into dead bee drawer (Masonite insert is placed over screen when not ineuse); B: double 5-mesh screen scrapes pollen pellets from bees' legs, allowing them to drop through 8-mesh excluder into pollen draw r (a 3-mesh screen is used when pollen is not being collected and an 18-mesh insert is used for closing hives when moving); C andoD: cleanouts for dead bee and pollen drawers which are removed from opposite side and are protected from weather by quarter-r und drip ledge; E: dead-bee pan, improved by attaching 2-mesh screen across top except for 9″×17″ opening around hive entrance.

Table 1.—Dead honey bees collected at hives after various treatments in repellency test, Pullman, Washington. 1959.

Treatment and Date	Dosage (lb./acre)	Total Bees in Trap Drawers and Pans for 2 Hives in Each Plot on Days After Application Shown						
		1	2	3	4	5	6	7
Applied before 7 a.m.								
Sevin 85% WP, July 1	1.5	948	434	91	48	30	20	15
Dieldrin 1.5 lb. E, July 1	0.5	4,332[b]	461	90	59	33	18	14
Sevin+R-874+Atlox 1045A, July 2	1.5 (10 oz., 1 oz.)	79[a]	18	19	14	6	18	14
Dieldrin+R-874, July 2	0.5 (10 oz.)	128[a]	5	37	27	17	12	8
Check, July 2	—	67[a]	9	29	17	11	13	11
Applied after 6 p.m.								
Sevin 85% WP, July 6	1.5	590	316	165	98	35	20	13
Dieldrin 1.5 lb. E, July 8	0.5	759	268	106	79	41	17	12
Sevin+R-874+Atlox 1045A, July 11	1.5 (10 oz., 1 oz.)	19	20	13	8	12	14	6
Dieldrin+R-874, July 11	0.5 (10 oz.)	21	19	12	5	10	14	10
Check, July 6	—	7	16	11	13	12	13	14

[a] Figures higher than normal because of first day after colonies were placed in the field.
[b] Derived by weight, 152 bees air dried 4 days = 10 grams.

collect bees dying within the hive (see fig. 1). The pans were similar to those described by Johansen *et al.* (1957). In samples of dead bees totaling 10,000, 80% were collected in the dead-bee pans and 20% in the trap drawers.

Bee activity in the plots was measured by taking 25 square-yard sight units each day after treatment for 7 to 9 days. The measuring instrument was of the type described by Smith & Townsend (1952). Dead bees which collected in the trap drawers and pans at the hives were counted each day after treatment for 7 days.

DISCUSSION.—Many dead honey bees were collected at the hives in fields treated with Sevin or dieldrin. Only approximately normal numbers were collected in the treatments combined with the repellent, R-874, and the

Table 2.—Bee foraging activity in blooming alfalfa fields after various repellency treatments, Pullman, Washington. 1959.

Treatment and Date	Maximum Temperature (°F.)[a]	Date Hives Placed in Field	Average Number Foraging Bees per 25 Sq.-Yd. Sight Units[b]									
			1 Day Before Application	On Days after Application								
				1	2	3	4	5	6	7	8	9
Sevin, July 1	71	June 26	19	—[c]	24	22	—	18	15	17	20	—
			16		14	14		12	19	12	15	
Dieldrin, July 1	71	June 26	18	—	22	23	—	17	14	16	21	—
			12		9	10		10	14	9	11	
Sevin+R-874+Atlox 1045A, July 2	71	July 1	11	0	0	—	0	0	0	2	—	8
			2	0	0		0	0	0	2		1
Dieldrin+R-874, July 2	71	July 1	17	0	2[d]	—	0	0	2	6	—	21
			4	0	0		0	2	2	3		3
Check, July 2	71	July 1	16	17	23	—	16	17	18	20	—	19
			4	4	5		7	4	3	4		2
Sevin, July 6	81	July 4	10	—	10	9	6	8	12	9		
			5		7	5	3	4	5	4		
Dieldrin, July 8	86	July 4	14	13	16	14	12	16	15	14		
			9	9	11	10	11	8	9	10		
Sevin+R-874+Atlox 1045A, July 11	87	July 8	10	0	0	1	6	8	8	7		
			4	0	0	2	7	5	4	5		
Dieldrin+R-874, July 11	87	July 8	15	0	1	2[d]	7	12	16	13		
			7	0	0	1[d]	6	6	8	6		
Check July 6	81	July 4	21	—	21	19	20	23	20	18		
			3		4	3	1	2	2	1		

[a] Average for the week after application.
[b] Upper figure is average honey bee count, lower is bumblebee count.
[c] No bees foraging because of inclement weather conditions.
[d] Bees observed did not forage normally and only for a short time in the alfalfa.

checks (see table 1). More dead bees were collected following treatments applied in the morning before 7 a.m. than from treatments applied in the evening after 6 p.m.

Bee foraging activity was not noticeably reduced in the Sevin- or dieldrin-treated fields as compared with the checks. Both bumblebees and honey bees were strongly repelled from the fields treated with insecticide-R-874 combinations (see table 2). Effective repellency lasted for 6 to 8 days when the average maximum temperature for the week following application was 71° F.; for 3 to 4 days when the average maximum temperature was 87° F. The Sevin-R-874 combination appeared to be slightly more repellent than the dieldrin-R-874 combination; however, no difference in protection against bee poisoning was shown.

Palmer-Jones *et al.* (1959) reported no evidence that R-874 repelled honey bees when applied mixed with Rogor (dimethoate) to a blooming turnip field. Several factors might account for this variation from our results. The application was made by aircraft at the rate of 7 gallons per acre; only 4 fl. oz. of R-874 were applied per acre; and the insecticide applied was an organic phosphate.

Dahmen & Menke (1959) also reported poor results with R-874 in treatments applied to blooming alfalfa in the Yakima Valley. Possible sources of variation in this case are: materials were applied by airplane; in all but one plot, only 5 fl. oz. R-874 were applied per acre; 1 fl. oz. of Plyac emulsifier (General Chemical Div.) per acre was used in all treatments; parathion or parathion and toxaphene were the insecticides mixed with R-874 in combination treatments; and the major test animal was the alkali bee, *Nomia melanderi* Cklle.

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Bee Poisoning Versus Clover Aphid Control in Red Clover Grown for Seed[1]

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Abstract

Twenty chemical treatments were tested for clover aphid (*Anuraphis bakeri* (Cowen)) control in red clover raised for seed in eastern Washington. Considering aphid control, safety to pollinators, and safety to aphid predators and parasites, the best materials were Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), phosphamidon, phorate, and Methyl Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate) plus Trithion. Treatments must be applied about the fourth or fifth week after the hay cutting is taken to obtain maximum effectiveness. Aphid damage to seed production involves both reduction in weight of individual seeds and reduction in number of seeds produced.

Studies of red clover pollination and seed production have been conducted in the Columbia Basin of Washington since 1956. During this time, the clover aphid (*Anuraphis bakeri* (Cowen)) proved to be consistently the most destructive pest. Satisfactory chemical controls for this aphid were previously unknown (Klostermeyer & Menke 1952, Portman & Barr 1952, Dickason & Every 1955). Field applications of parathion, or demeton, to control this pest had not been effective in trials during 1953–55 (Johansen 1954). Trithion[2] and Tetram were tested for controlling aphids in 1957. Although these materials were also unsatisfactory, Trithion did give a spectacular kill of aphids that were not protected in the clover heads. All of these trials were conducted during July when the clover was well into the blooming period. It appeared that earlier applications of insecticides, as the aphids were moving from the stipules to the heads, might be successful. Since applications would be made on a blooming crop, it was also important to assess the relative bee poisoning hazard of the materials tested.

CLOVER APHID CONTROL, 1958.—Experimental plots were established in a 20-acre field of red clover raised for seed near Quincy, Washington. Each treatment was replicated four times and each replicate was one-eighth acre in size. All treatments were applied June 27, about 5 weeks after the hay cutting. Nine insecticidal materials were applied with a tractor-drawn, horizontal boom sprayer. The sprayer was operated at 30 p.s.i. pressure and 24 gallons of liquid were applied per acre.

The first aphids observed in the Quincy area in 1958

[1] Scientific paper 1950. Washington Agricultural Experiment Stations. Work conducted under Project No. 1419 and 1426, partially supported through grants from California Spray-Chemical Corp., Shell Chemical Corp., and Stauffer Chemical Company. Accepted for publication May 10, 1960.

[2] The chemical names of insecticides mentioned in this paper that do not have common names adopted by the Entomological Society of America are given in the paragraph preceding "References Cited."

Table 1.—Control of clover aphid in red clover raised for seed, Quincy, Washington, 1958.

Material and Pounds per Gallon of Emulsifiable	Dosage (lb./a.)	Clover Aphids After Treatment (four 10-head samples) 6 Days: Total Living	6 Days: No. Heads Infested	18 Days: Total Living	18 Days: No. Heads Infested
Demeton, 2	0.5	8	3	592	39
Guthion, 1.5	1.0	23	12	744	39
Phosdrin, 4	0.5	23	5	604	38
Phorate, 2	0.5	0	0	219	32
Dibrom, 8	1.0	55	12	612	38
Trithion, 4	1.0	11	4	69	17
Thiodan, 2	1.0	21	7	461	33
C-140, 5.6	1.4[a]	160	13	721	38
Sevin, 2.5[b]	2.0	3	1	220	34
Check		315	38	1,939	40

[a] Plus 4 ounces Triton B-1956, Rohm & Haas Co.
[b] Paste formulation.

were on volunteer clover plants adjacent to the experimental field 16 days before the application. Eight days before treatment the infestation averaged 16 per 10 stipules in the experimental field and a few were moving into the flower buds. There was an average of 37 red blooms per square yard. The infestation averaged 50 per 10 heads at the time of application and there were 106 red blooms per square yard on the average. Results of this test are given in table 1. Ten heads were collected in each replicate, so the figures listed represent the total number of living aphids and number of heads infested per 40 heads examined. Differences in seed yields in six of the treatments and in the check are shown in table 2.

BEE POISONING, 1958.—Two bee poisoning tests were conducted in a field of red clover raised for seed near Pullman, Washington. Two package-bee cages containing 50 to 100 honey bees were placed in each plot immediately before spraying. Each treatment was replicated four times as two cages in each of two plots per material. Each plot was one-tenth acre in size and the first test was started August 4, the second, August 19. All treatments were applied with a truck-mounted, horizontal boom sprayer. The sprayer was operated at 125 p.s.i. liquid pressure and 15 gallons of spray mixture were applied per acre.

After treating, the cages of bees were removed to a holding room (temperature about 75° F.), fed 1:1 sugar-water syrup, and held for 24-hour mortality counts. At intervals after spraying, bouquets of red clover from each plot were placed in two unexposed package-bee cages.

Table 2.—Red clover seed yields in clover aphid control plots, Quincy, Washington, 1958.

Treatment	Clean Seed[a] (lb./a.)	Increase Compared with Check (%)	Clean Seed[b] (lb./a.)
Demeton	815	5.4	419
Phorate	871	12.6	448
Trithion	886	14.5	456
Thiodan	832	7.6	428
C-140	719	—	398
Sevin[c]	871	12.5	448
Check	774	—	398

[a] Yields calculated from 3 square-yard samples from each treatment.
[b] Yields calculated from harvest data for entire field, 8061 lb. from 20 acres.
[c] Paste formulation.

Table 3.—Bee poisoning in red clover raised for seed. Pullman, Washington, 1958.

Material (Emulsifiable)	Dosage (lb./a.)	Honey Bee 24-Hour Mortalities (%) Sprayed During Application	5-Hr. Residual	1-Day Residual	2-Day Residual	4-Day Residual	7-Day Residual
Demeton	0.5	28	0	1	—	—	—
Guthion	1.0	100	43	8	4	4	0.3
Phosdrin	0.5	100	6	0	—	—	—
Phorate	0.5	100	24	0	0	—	—
Dibrom	1.0	100	7	0	—	—	—
Trithion	1.0	100	4	0	—	—	—
Thiodan	1.0	24	3	0	—	—	—
C-140	1.4	23	0.8	1	—	—	—
Sevin[a]	2.0	69	23	9	15	13	4
Ethion	0.5	2	0.9	0.8	—	—	—
Phosphamidon	0.5	76	0	0	—	—	—
Check		0.3	0	0	0.7	0	0

[a] Paste formulation.

Each cage was loaded with 50 to 100 honeybees and held as indicated above. Results of these tests are shown in tables 3 and 4.

CLOVER APHID CONTROL, 1959.—Clover aphid plots were established in a field of red clover raised for seed near Quincy, Washington. Each treatment was replicated four times and each replicate was 1/100 acre in size. All treatments were applied June 23, about 5 weeks after the field had been pastured off with sheep. Treatments were applied with a hand sprayer, using 20 p.s.i. pressure and applying 25 gallons per acre. Granular formulations were applied with a rotary hand granule spreader.

Two weeks before treatment, the clover aphid infestation averaged 6 per 10 stipules and 3 per 10 heads. There was an average of three red blooms per square yard at that time. The infestation averaged 20 to 25 per 10 heads at the time of application and there were 50 red blooms per square yard on the average.

Results of this test are given in table 5. Differences in seed yields among seven of the treatments and the check are shown in table 6.

BEE POISONING, 1959.—A bee poisoning test was conducted in a field of red clover raised for seed near Pullman, Washington. Each treatment was replicated four times as two cages in each of two plots per material. Each plot was 1/100 acre in size and the test was started on August 11. Applications were made with a hand sprayer, using 20 p.s.i. pressure and applying 25 gallons per acre. Granulated formulations were applied with a rotary hand granule spreader.

Caged bees were handled as described for the 1958

Table 4.—Bee poisoning in red clover raised for seed. Pullman, Washington, 1958.

Material (Emulsifiable)	Dosage (lb./a.)	Honey Bee 24-Hour Mortalities (%) Sprayed During Application	5-Hr. Residual	12-Hr. Residual	1-Day Residual
Phosdrin	0.5	100	8	1	0
Phorate	0.5	100	10	0	0.3
Dibrom	1.0	100	7	0.6	0
Trithion	1.0	100	8	3	0
Check		0	0	0	0

Table 5.—Control of clover aphid in red clover raised for seed. Quincy, Washington. 1959.

Treatment	Toxicant (lb./a.)	Clover Aphids After Treatment (four 10-head Samples)					
		1 Day		6 Days		18 Days	
		Total Living	No. Heads Infested	Total Living	No. Heads Infested	Total Living	No. Heads Infested
Endrin 1.6 lb. E.	0.2	36	12	153	30	1,121	40
Phorate 5%, granules	1.0	5	3	17	7	180	31
	2.0	1	1	4	3	113	23
Ethion 4 lb. E.	0.5	41	6	78	16	172	30
Phostex 8 lb. E.	1.0	92	20	129	26	1,548	40
Phosphamidon 4 lb. E.	0.5	23	5	6	4	179	35
	1.0	4	3	3	3	79	14
Dimethoate 4 lb. E.	1.0	29	5	5	3	210	33
Di-Syston 5%, granules	1.0	36	7	86	17	281	34
Methyl Trithion 4 lb. E.	1.0	22	8	56	11	137	23
Methyl Trithion +Trithion 4 lb. E.	1.0	88	12	39	10	76	19
Check	—	89	19	218	28	1,657	40

Table 6.—Red clover seed yields in clover aphid control plots. Quincy, Washington. 1959.

Treatment	Clean Seed[a] (lb./a.)	Increase Over Check (%)	Clean Seed[b] (lb./a.)
Endrin E.	431	—	341
Phorate granules (2 lb.)	569	28	336
Ethion E.	517	16	396
Phosphamidon E (1 lb.)	618	39	474
Dimethoate E.	528	19	404
Methyl Trithion E.	527	18	403
Methyl Trithion+Trithion E.	594	33	454
Check	445	—	341

[a] Yields calculated from 3 square-yard samples from each treatment.
[b] Yields calculated from harvest data for entire field, 9540 lb. per 20 acres.

tests, except in granule treatments. In these plots, a piece of paper was attached across the bottom of each cage. Therefore, the granules falling through the top screen remained between the paper and the bottom screen of the cage. This paper was removed 2 hours after application of the insecticide and the cage placed upright with a clean syrup feeder on top. Results of this test are listed in table 7.

Effect on Beneficial Insects.—Predators and parasites of the clover aphid in the control plots were sampled after the applications. The most prevalent species were the convergent lady beetle (*Hippodamia convergens* G.-M.), the pirate bug (*Orius tristicolor* (White)), the big-eyed bug (*Geocoris pallens* Stal.), the syrphid fly (*Scaeva pyrastri* (L.)), the eulophid parasite (*Aphelinus lapisligni* How.), the lacewing (*Chrysopa plorabunda* Fitch), and the damsel bug (*Nabis alternata* Parsh.). During the first six days after application, endrin, Phostex, C-140 Guthion, phosphamidon, Dibrom, Trithion, and Methyl Trithion plus Trithion (in that order) were the least destructive to the beneficial insects. These materials reduced predator-parasite populations 38% to 67% as compared with the checks.

Timing of Applications for Clover Aphid Control.—The clover aphid begins moving from the stipules to the heads of red clover 3 to 4 weeks after the hay cutting is taken in Columbia Basin seed fields. Applications must be made about the fourth or fifth week after cutting in order to gain maximum effectiveness. Furthermore, if a

Table 7.—Per cent honey bee mortalities from treatments on red clover. Pullman, Washington, 1959.

Treatment	Toxicant (lb./a.)	Sprayed During Application (cumulative % mortalities)				Caged with Treated Foliage[a]	
		1 Hr.	3 Hr.	10 Hr.	24 Hr.	2 Hr.	1 Day
Endrin E.	0.4	64	80	100	—	3	1
Phorate granules	2.0	0[b]	42	96	100	2	1
Ethion E.	1.0	47	100	—	—	1	0
Phostex E.	1.0	37	91	98	100	13	0
Phosphamidon E.	1.0	56	100	—	—	7	1
Dimethoate E.	1.0	78	100	—	—	100	1
Di-Syston granules	2.0	0[b]	0	0	3	1	2
Methyl Trithion E.	1.0	55	100	—	—	99	1
Trithion+Methyl Trithion E.	1.0	58	97	99	100	79	0
Phosdrin E.	0.5	100[c]	—	—	—	2	2
Trithion E.	1.0	60	84	100	—	79	0
Check	—	0	0	1	2	1	0

[a] Two hours or 1 day after application of insecticide. Per cent mortality determined 24 hours later.
[b] Bees showeddistinct agitation prior to removal of paper retainers after 2 hours.
[c] 100% mortality within 15 minutes with bees typically extruding tongues and releasing drops of clear fluid upon them.

hay cutting is not taken, the aphid treatment cannot be effectively timed.

During 1959, a seed grower in the Quincy area did not take a hay cutting from one of his fields. Subsequent small samples and harvest results indicated that there was about a 25% reduction in yield by weight which could be attributed to poor aphid control as compared with clipped fields.

Table 8 presents criteria for timing and determining the necessity for applying treatments. Our studies show that aphid development will follow these patterns during most seasons. Two seasons of field experience in the Columbia Basin and the Yakima Valley have given good results with this timing schedule (Johansen 1960).

CLOVER APHID DAMAGE TO SEED.—Weights of 1,000 seed subsamples were obtained from various red clover plots during 1957 and 1958. These data indicate that 6.5% to 8.8% of the reduction in seed yields by clover aphid can be caused by a reduction in weight of individual seeds. It was determined that about 60% of the seed loss from the aphid is caused by reduction in weight and 40% to reduction in number of seeds. Injured seeds are difficult to distinguish from noninjured seeds with the unaided eye. They appear slightly shriveled—slightly less plump than normal. Germination tests show that aphid-injured and normal seeds have equal germinating quality.

CHEMICAL DEFINITIONS.—The chemical definitions of the insecticides mentioned in this paper that do not have adopted common names are as follows:

C-140, dimethylaminomethylphenyl *N*,*N*-dimethylcarbamate hydrochloride.

Dibrom®, 1,2-dibromo-2,2-dichloroethyl dimethyl phospate.

Di-Syston®, *O*,*O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate.

Guthion®, *O*,*O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate.

Methyl Trithion®, *S*-(*p*-chlorophenylthio)methyl *O*,*O*-dimethyl phosphorodithioate.

Table 8.—Criteria for determining severity of infestation by clover aphids based on number of aphids per ten heads or stipules.[a]

EXTENT OF INFESTATION	WEEKS AFTER HAY CUTTING				
	3	4[b]	5[b]	6	7
Light	0– 5	0–10	0– 15	0– 50	0–150
Medium	10–25	15–50[c]	20–100[c]	60–250	200–750
Heavy	25+	75+	200+	500+	1,000+

[a] Based on populations in central Washington, 1956–59.
[b] Aphids moving from stipules to heads of clover plant.
[c] Insecticide treatment recommended if medium or heavy population is developing.

Phosdrin®, 1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate.

Phostex®, a mixture of bis(dialkyloxyphosphinothioyl) disulfides.

Sevin®, 1-naphthyl methylcarbamate.

Tetram®, *O*,*O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate [hydrogen oxalate salt].

Thiodan®, 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2-4-3-benzodioxathiepin-3-oxide.

Trithion®, *S*-(*p*-chlorophenylthio)methyl *O*,*O*-diethyl phosphorodithioate.

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Soil Insects in Hokkaido, Japan, with Special Reference to the Effects of Some Chlorinated Hydrocarbons[1]

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ABSTRACT

On the island of Hokkaido there are many soil-insect pests, of which the most important are the seed-corn maggot (*Hylemya ciliercura* (Rond.) (*platura* (Meig.)), onion maggot (*H. antiqua* (Meig.), turnip maggot (*H. floralis* (Fall.)), cutworms, chafers, wireworms, and a mole cricket. The control of these pests is difficult owing to their subterranean habits. In recent years chlorinated hydrocarbon insecticides have given improved control. Heptachlor and aldrin are most desirable because of uniform effectiveness, low price, and ease of use.

As in the southern parts of Japan and other countries in the world, soil insects are injurious to upland crops in Hokkaido, the northernmost island of Japan. Maggots, mole crickets, cutworms, wireworms, and chafers cause the most severe injury, and control has been especially

[1] Accepted for publication April 28, 1960.
[2] The authors are grateful to Dr. C. H. Hoffmann of the U. S. Department of Agriculture and to Mr. S.S. Easter of the Velsicol International Corporation for their valuable advice in the preparation of this paper.

Table 1.—Per cent of kidney-bean seed injured by the seed-corn maggot as affected by insecticide dusts used as seed coatings, mixed with soil in the furrows, and mixed with fertilizer. Fall 1956.

Insecticide	Seed Coating			In Furrows			With Fertilizer
	0.3%	0.5%	1%	9 Lb./Acre	18 Lb./Acre	27 Lb./Acre	27 Lb./Acre
BHC, 3% gamma	100	70	66	16	17	17	18
1.5%	93	81	86	39	37	9	17
Aldrin 4%	79	75	36	24	19	14	18
Dieldrin 4%	74	78	79	12	23	13	11
Heptachlor 15%	23	20	19	—	—	—	—
4%	74	68	42	18	18	14	19
2.5%	79	61	31	22	19	9	12
2%	87	57	58	28	19	17	16
1.5%	91	89	62	20	23	23	22
EPN 1.5%	78	80	86	16	26	31	21
Check	94	100	80	93	81	77	93

difficult owing to their subterranean habits. The authors have studied the life histories and habits of these pests and experimented with control measures for several years, and in recent years have obtained good results with some of the chlorinated hydrocarbons.

Seed-Corn Maggot.—This widely distributed maggot, *Hylemya cilicrura* (Rond.) (*platura* Meig.), has caused severe injury to kidney bean since about 1917, when its cultivation was greatly expanded owing to the sudden rise in price. Since that time this pest has been a problem not only on kidney bean but also on soybean, Adzuki bean, melons, asparagus, wheat, barley, Indian corn, and other crops.

Based on the seasonal prevalence and habits of this maggot and on experiments conducted since 1929, recommendations for its control have been (1) regulation of the sowing period, especially early sowing during the first 20 days of May in the case of kidney bean; (2) covering seed with soil immediately after sowing; (3) avoiding the use of fish cake, fresh compost, or night soil; (4) removing injured seeds or young seedlings in small fields; and (5) coating seeds with insecticides or mixing insecticides in sowing furrows (Kuwayama & Sakurai 1957). The authors' experiments with chlorinated hydrocarbons since 1955 reveal that the seed coating is inferior to mixing in the furrows. Aldrin, dieldrin, and heptachlor are effective and not phytotoxic. BHC is less effective and is harmful to seedlings in some cases. By mixing with fertilizer the effectiveness is not reduced. Granules are suitable for this purpose. In 1957, 15% heptachlor granules proved nearly equal to 2.5% heptachlor dust at the same dosage of active ingredient. Heptachlor and aldrin dusts had residual effectiveness for more than 100 days (Endo *et al.* 1957b). This method was recommended by the Extension Service of the Hokkaido Prefectural Office, and has been used by farmers. The results of tests against the seed-corn maggot are presented in tables 1, 2, and 3.

Onion Maggot.—Serious injury by this maggot, *Hylemya antiqua* (Meig.), was first noticed in 1938 at Furen, Kamikawa District, in the northern part of Hokkaido. Since that time the infested area has greatly increased and the pest is now present all over the island. Since 1949 outbreaks have been recorded from Honshu, Kyushu, and Shikoku. Both onion and Welsh onion were infested. When this maggot began to spread, no effective insecticides were known; even DDT was ineffective. It was therefore recommended that it be controlled by (1) eliminating overwintered puparia, (2) fall plowing, (3) eliminating the infested seedlings, and (4) the use of transplants as the injuries to plants from direct sowing are more severe (Endo *et al.* 1953).

Table 2.—Per cent of kidney-bean seed injured by the seed-corn maggot following broadcast treatments with 44.5 pounds of 4% aldrin or heptachlor dust on May 7, 1957.

Insecticide	May 7	June 6	July 10	Aug. 16	Sept. 5	Sept. 22
Aldrin	0	2.8	2.8	3.7	13.9	81.1
Heptachlor	1.7	2.2	2.1	1.1	8.3	77.3
Check	15.0	97.2	92.7	65.0	100.0	88.0

In 1952 BHC was found to control this pest. Since then many experiments have been made with other chlorinated hydrocarbons. Mixing heptachlor or aldrin dusts with the soil in furrows just before sowing is a satisfactory practice. In onion culture where the distance between the furrows is 18 inches, 2.14 pounds of aldrin or 1.34 pounds of heptachlor per acre is required. In Welsh onion culture the distance between furrows is 39 inches; therefore the dosage of either insecticide can be reduced to 1.07 pounds per acre. The application of heptachlor or aldrin in the onion fields is now extending remarkably. The results of tests against the onion maggot are presented in table 3.

Turnip Maggots.—The common turnip maggot (*Hylemya floralis* (Fallén)) has been one of the major insect pests on the island for a long time, damaging daikon (large white radish), rutabaga, cabbage, Chinese cabbage, turnip, and other crucifers. This pest had been known as *Anthomyia flavopicta* Matsumura, but Kato determined it to be *H. floralis* (Fallén) in 1939. Though its distribution in Japan is limited to Hokkaido, the injuries are serious, the infestation of daikon being 100% on occasion (Kato 1958).

This maggot produces one generation each year, but in 1938 the senior author observed another closely related maggot which has three generations per year in the cooler districts such as Nemuro Subprefecture. It was identified

Table 3.—Effect of mixing aldrin or heptachlor dusts with soil in furrows on injury by the seed-corn maggot to kidney beans and the onion maggot to onion, 1958.

Insecticide	Seed-Corn Maggot		Onion Maggot	
	Toxicant per Acre (lb.)	Injured Seeds (%)	Toxicant per Acre (lb.)	Injured Seedlings (%)
Aldrin 1%	0.27	0.5	—	—
2%	.54	.5	1.07	0
2.5%	.67	1.5	1.34	0
4%	1.07	1.0	2.14	0.7
Check	—	—	—	70.6
Heptachlor 1%	0.27	0.5	—	—
2%	.54	1.6	1.07	0
2.5%	.67	0	1.34	0
4%	1.07	2.1	2.14	0
Check	—	47.1	—	34.3

by Kato as *Hylemya pilipyga* (Villeneuve). This smaller turnip maggot is found on about the same hosts as *floralis* (Kato 1958).

On account of severe injuries of the turnip maggot, various control experiments were conducted for many years. Prior to about 1950, spraying 0.05% to 0.1% mercuric chloride so that it came in contact with roots near the surface of a daikon field during the oviposition period was recommended. It must be applied three to five times from about the middle of August at intervals of 7 to 10 days at the rate of 107 to 160 gallons per acre.

In recent years chlorinated hydrocarbons have shown greater effectiveness than mercuric chloride. Aldrin and heptachlor are equally effective and economical when mixed with soil or fertilizer in the furrows just before sowing (table 4). Experiments on the depth of placement (table 5) show that scattering at a 2-inch depth is most effective, followed by mixing with top soil. Since 2 inches is the depth that seeds are placed, the insecticide may be applied to the furrows just before sowing, or may be mixed with fertilizer. The farmers are now taking up this practice and are satisfied with the results (Endo *et al.* 1957a).

CUTWORMS.—In Hokkaido many species of cutworms are pests of sugar beets, flax, legumes, onion, cabbage, and other crops. *Agrotis ipsilon* Hufn., *A. fucosa* Butl., *A. exclamationis informis* Leech, and *Amathes c-nigrum* (L.) are common and devour crops ravenously on occasion. Former recommendations were the application of poison bait, trapping moths by molasses specially prepared, and regulation of transplanting time of some crops. However, as shown in table 6, chlorinated hydrocarbons will also control cutworms. Mixing heptachlor or aldrin dust with the soil in furrows just before sowing or in holes just before transplanting will prevent attacks.

CHAFERS.—Formerly only the infestation of rangeland, roadsides, and idle and waste land by the larvae of Japanese beetle (*Popillia japonica* New.) was known in Hokkaido, but in 1929, following reclamation of the volcanic-ash soil of Nemuro Subprefecture in the southeastern part of the island, an unexpected severe damage by chafers was first noticed. The underground parts of almost all upland crops and weeds were infested, the roots being frequently cut off. The species was identified by the senior author as *Mimela testaceipes* Motschulsky. Severe injury by the larvae of this species and also of *Anomala lucens* Ballion and *A. rufocuprea* Motschulsky continued for about 10 years, though yearly fluctuations were noticed. The infestation spread over the southern half of the island, where the volcanic-ash soil is widely distributed. In the last 10 years, however, such severe injury has not been noticed in these areas (Kuwayama 1937).

Previous recommendations for control of these chafers were (1) crop rotation, (2) summer plowing, (3) collection of adult beetles and larvae, (4) light traps for adults, (5) application of some insecticides, and (6) protection of natural enemies. Collection of adults by hand, digging out of larvae, and trapping of adults by blazing fire were practiced during the years of severe outbreaks, and were partially effective, but these methods were laborious. Spraying with lead arsenate was found to be effective against the adults, but putting lead arsenate into top soil was not effective against larvae and, moreover, phytotoxicity to oats and Adzuki bean continued for several years (Kuwayama 1937). In experiments at the Nemuro Branch Station in 1942 disking three or four times after plowing in the spring killed 26% to 37% of the larvae of *Mimela* and *Anomala* spp. Since the population has decreased in recent years, the merit of new synthetic insecticides such as chlorinated hydrocarbons has not been detected.

WIREWORMS.—The serious damage by wireworms to cereals and potatoes has been known for a long time in

Table 4.—Effectiveness of aldrin and heptachlor mixed with soil or fertilizer in the furrows against the turnip maggot on daikon, 1957 and 1958.

INSECTICIDE	1957 TESTS				1958 TESTS	
	Toxicant per Acre (lb.)	Per Cent of Injured Roots			Toxicant per Acre (lb.)	Injured Roots (%)
		Main Station	Kotoni	Sorachi Branch		
Dusts:						
Aldrin 2%	1.07	13.9	—	7.0	1.06	47.6
2.5%	—	—	—	—	1.32	32.7
3%	—	—	—	—	1.59	22.4
4%	2.14	4.4	2.8	2.2	2.12	20.9
Heptachlor 2.5%	1.34	2.2	0.4	4.2	1.32	16.0
Check	—	71.3	23.2	16.2	—	95.9
Heptachlor 15% granules	1.32	4.3	—	1.6	—	—
Aldrin 0.6% with fertilizer	1.11	37.4	—	—	—	—
Fertilizer only	—	85.2	—	—	—	—

Table 5.—Relation between depth of placement of aldrin and heptachlor dusts to damage of the turnip maggot on daikon, 1958.

INSECTICIDE	TOXICANT PER ACRE (LB.)	DEPTH OF PLACEMENT	INJURED ROOTS (%)
Aldrin 4%	2.12	Surface	82.0
		2 inches	22.5
		4 inches	83.8
		Mixed with top soil	37.2
Heptachlor 2.5%	1.32	Surface	89.2
		2 inches	17.2
		4 inches	72.8
		Mixed with top soil	19.4
Untreated			90.0

Table 6.—Effect of mixing heptachlor and aldrin dusts with soil in furrows on damage to daikon caused by the cutworm, *Agrotis fucosa*, 1958.

INSECTICIDE	TOXICANT PER ACRE (LB.)	INJURED (%)	MARKETABLE (%)
Heptachlor 2.5%	0.45	50.7	60.0
	.67	49.3	65.4
	.89	34.7	78.6
Aldrin 4%	1.07	36.0	77.3
Check	—	65.3	41.4

Table 7.—Effect of broadcasting insecticide dusts over the field on damage to potatoes by wireworms and mole cricket. 1956.

Insecticide	Toxicant per Acre (lb.)	Per Cent of Injured Tubers by	
		Wireworms	Mole Cricket
Heptachlor 2.5%	1.1	3.2	0.3
	2.2	1.6	.1
	3.3	2.5	—
Aldrin 4%	1.8	4.3	0.2
BHC 3% gamma	0.8	2.8	—
EPN 1.5%	0.7	—	9.5
Check	—	40.7	10.6

Hokkaido. The species was first thought to be *Agriotes sericeus* Candèze, but the second author (Sakurai) found eight injurious species—*Lacon binodulus* Motschulsky, *Melanotus caudex* Lewis, *M. legatus* Candèze, *Ctenicera puncticollis* Motschulsky, *Agriotes fuscicollis* Miwa, *A. persimilis* Lewis, *Silesis musculus* Candèze, and *Platynychus* sp?. *A. fuscicollis* is found in the ill-drained peat soil, being restricted chiefly to the Ishikari basin. On the contrary, *M. caudex* occurs chiefly in light volcanic-ash soil, but is sometimes found in clay or clay loam, and is widely distributed in the Ishikari plain and the Iburi, Tokachi, Kamikawa, and Abashiri Subprefectures. *C. puncticollis* is limited to clay soil in the northern part of the Sorachi Subprefecture. *A. fuscicollis* causes the greatest damage to crops. *M. caudex* may be ranked second. *C. puncticollis* is probably third because of its limited distribution, but its feeding potential is greater than that of the other two; sometimes 50% to 80% of potato tubers are damaged and there is 12% to 15% loss in yield. The remaining species have no economic importance (Sakurai 1952).

The third author (Endo) classified the crops infested by wireworms, especially by *A. fuscicollis*, as follows, according to their severity: (1) oats, barley, naked barley, wheat, Indian corn, potatoes, turnip, carrot, grasses; (2) sugar beet, cabbage, Chinese cabbage, soybean, Adzuki bean, peas, Welsh onion; (3) kidney bean, broad bean, buckwheat, cucumber, eggplant, tomato. Of course, these classes vary considerably with the population density of wireworms, cultivated areas of crops, and other environmental factors. Buckwheat, flax, and rape are the least infested, followed by legumes, cucumber, tomato, eggplant, and edible root burdock. It is reported that buckwheat and flax are resistant to the wireworms in North America and Europe.

The following control methods have been suggested: rotation of crops, change of sowing time, soil improvement, summer plowing, and trapping. Endo observed the effect of food trapping on the larvae of *A. fuscicollis* in 1945. Traps baited with pieces of sugar beet, potato, and carrot were set in the soil between the rows and were effective when used 3 days before sowing. He also attracted hibernated adults by the use of semidried clover or chickweed.

BHC, aldrin, and heptachlor have been found effective against wireworms. Control on oats and other grain crops by the seed treatment is easy, but the common method, especially in potato, is broadcasting the insecticides over the field just before planting. BHC is recommended for use at the rate of 67 to 89 pounds of a 0.5% or 13 to 18 pounds of a 3% gamma dust per acre. However, BHC dust tends to be toxic to vegetation in time. At present aldrin and heptachlor are used at the respective rates of 1.8 and 1.1 pounds per acre (table 7). Recent experiments reveal that the dosage of aldrin can be lowered to that of heptachlor (Sakurai *et al.* 1957).

MOLE CRICKET.—The damage to wheat and barley done by the mole cricket (*Gryllotalpa africana* (P. de B.)) has been known for a long time. Recently severe damage to potatoes was noticed in the seed-potato areas in the Oshima, Hiyama, Shiribeshi, Iburi, Ishikari, Sorachi, and Kamikawa Subprefectures. Sometimes more than 50% of the tubers were injured (Sakurai *et al.* 1955). Experiments since 1953 showed that aldrin and heptachlor were excellent for the control of this mole cricket (table 7). The recommendation is to broadcast 4% aldrin or 2.5% heptachlor dust at the rate of 27 to 45 pounds per acre over the field and work into the top 4 inches of the soil just before sowing time in May. It is also effective to mix these insecticides with the soil just before soil dressing during July. These chlorinated hydrocarbons do not seem to be ovicidal, but are highly effective against nymphs and adults and have a long residual effect.

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[3] All are written in Japanese, except Kato (1958).

DDT Residues on New York Dairy Farms Following the Gypsy Moth Eradication Program[1]

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Abstract

Aerial-applied DDT-oil solutions for eradication to the gypsy moth, *Porthetria dispar* (L.), in 1957 were found to be highly persistent on forage and little affected by weathering. However, the growth of new grass with its great increase in mass and volume was found to be a very important factor in residue "loss."

The use of DDT as an eradicant spray resulted in widespread contamination of milk which persisted for at least 1 year. At one poultry farm checked, eggs were found to be contaminated for a period of 1 month.

During the winter of 1956, the United States Department of Agriculture (USDA), in cooperation with state agencies, announced plans to aerial spray nearly 3 million acres of forested land in the Northeast to eradicate the gypsy moth, *Porthetria dispar* (L.). Of this area, an estimated 2,540,000 acres were in New York. Since the program was one of eradication, it called for a thorough coverage of all host plants, whether trees or shrubs in forests or shade trees and foundation plantings in urban and farming areas. DDT, which had been used effectively for 10 years in controlling the gypsy moth caterpillars in forested areas, was chosen as the most effective and suitable insecticide. The DDT was to be applied from the air as an oil solution at the rate of 1 pound of actual toxicant in 1 gallon of mixed oil solvents per acre. As previous control programs for gypsy moth caterpillars were conducted primarily in wooded areas, information was lacking on insecticide residues from such a program in dairy farm areas. Research, therefore, was initiated in New York in cooperation with the USDA late in the winter of 1956. The research program was divided into several phases. The first experiments were early trials outside of the infested areas and were designed to determine the magnitude of residues one might expect from aerial spraying of the DDT solution on hay, and its breakdown, through normal process of weathering as well as the effects of dilution by growth. Later tests were designed to sample DDT residues of meadows and pastures under actual conditions of aerial spraying and to follow these insecticide residues throughout the year as they might be dissipated by weathering and growth, in storage, and to study the residues, if any, in the milk of dairy cattle and eggs of poultry on several farms in the treated areas. In addition, it was deemed advisable to sample for DDT residues in milk at processing plants following a general mixing of the milk from treated and untreated areas.

Methods.—In the first preliminary experiment, a level field planted to mixture of meadow grasses which had grown to a height of 5 to 7 inches in 1956, but had not been harvested, was divided into ten ⅛-acre plots. These plots were arranged in two adjacent rows of five each. Prior to spray application grass samples were harvested from 10 arbitrarily chosen 1-square-foot areas in each plot. The samples for each plot were composited and subsampled for residue analysis. These pretreatment samples were to serve as untreated checks. On the evening of March 18 during a light wind of 5 to 7 m.p.h., the plots were treated with a No. 135 Piper Cub flown at a height of about 20 feet. The airplane was to have applied 1 pound per acre of toxicant but because of an error in calibration actually applied 2.6 pounds per acre.

Following treatment, 10 additional samples of grass were taken by means of a Power Scythette Scythe in random chosen locations from each plot at 0-, 7-, and 49-day intervals after application. These samples for each plot at each sampling interval were composited and subsampled for chemical analysis. After the grass samples chosen for analysis had been stripped of their residues, analysis for DDT was made by the method of Downing & Norton (1951).

Since there had been an error in calibration of the spray plane in Experiment I, an additional experiment, which can be designated as Preliminary Expt. II similar in size and design to the previous one, was laid out and treated. In Experiment II, grass samples were taken at 0-, 7-, and 98-day intervals after application. Unlike the earlier test, it was determined that the desired dosage was applied.

For a study of the residues of DDT in milk, eggs, and on hay and pasture under the actual spraying operation, an area near Norwich, New York comprising parts of five townships in the Chenango Valley was chosen. This area which is an important milk-producing area consists of rolling woodlands, intersperced with open cultivated valleys of meadows and pastures. In this area near Norwich five dairy farms and one commercial poultry farm were chosen. For the purposes of this paper these henceforth will be referred to, arbitrarily, as Farms A, B, C, D, E, and F. Four other farms, arbitrarily called G, H, I, and J, were chosen as supplementary sampling sites outside of the test area but in the gypsy moth-treated areas on Long Island and in southern New York.

At all of these locations prior to DDT treatment, samples of grass were taken in a manner previously described and periodically thereafter similar samples of the DDT-treated forage were taken at varying intervals. Milk samples which consisted of 1 pint of well mixed raw milk were taken prior to any spraying and periodically thereafter for 1 calendar year. In addition to these farm samples, others of similar size were taken at similar sampling intervals at two milk plants which obtained their milk from farmers both within and outside the treated area.

Discussion.—Although precipitation measured 4.71 inches in the 7 days following treatment of Experiment I, including 18.75 inches of snow, it seems obvious from the data given in table 1 that there was little or no loss of DDT residues that could be attributed to weathering.

[1] Accepted for publication February 29, 1960.

[2] The authors acknowledge with thanks the assistance of R. L. Ridgway and B. D. Hilton of Cornell University and W. V. O'Dell, A. Higgins, and E. D. Eckess of the U.S.D.A. in some phases of the work.

This work was in part supported by funds from the Northeast Regional Project NE-36.

Table 1.—Residues on meadow grass[a] at various time intervals following aerial application of 2.6 pounds of DDT per acre in an oil solution of mixed solvents. Ithaca, New York, March 18, 1957.

Plot No.	Residues in P.P.M. at Days Shown		
	0	7	49
1	451.8	339.1	31.2
2	156.5	170.4	14.6
3	395.8	252.0	49.7
4	96.8	123.6	8.4
5	386.0	312.0	27.5
6	74.2	88.8	7.0
7	259.5	253.2	26.3
8	73.0	75.6	5.9
9	207.4	279.6	30.3
10	69.4	61.2	5.4

[a] Dry weight.

With new growth, residue dilutions were rapid and great, indicating that residue loss can best be brought about by early application prior to growth of forage. New growth is extremely important in lowering residues. It should also be noted (table 1) that the odd-numbered plots which were to the windward side of the even numbered plots received an additional dosage of DDT from drift.

The data given in table 2 are very similar to those in table 1 and again show the persistence of residues from the DDT-oil solution and the importance of new growth on residue loss. The residues from the 1-pound dosage of DDT are also extremely high and persistent.

The residues under actual spray conditions at Norwich are like those for the preliminary experiments except there was greater variation between farms than between the two trial experiments (See table 3). Unlike the earlier work the data for pastures are presented on a green weight basis. These may be corrected by an approximate 4 to 5× factor to be comparable to the previous tables. It should also be noted that in spite of dilutions by growth and weathering some residues persisted throughout the entire sampling period.

The applications of DDT made in the Norwich area in 1957 were delayed by bad weather and various mechanical difficulties with the aircraft and were not made until hay was being harvested. It is not surprising, therefore,

Table 2.—Residues on meadow grass[a] at various time intervals following aerial application of 1.0 pound of DDT per acre in an oil solution of mixed solvents. Ithaca, New York, April 13, 1957.

Plot No.	Residues in P.P.M. at Days Shown		
	0	7	97
1	24.4	53.9	0.6
2	124.2	103.7	—
3	113.6	80.9	0.6
4	108.2	65.9	0.0
5	337.2	179.9	—
6	222.2	200.3	0.0
7	261.2	199.7	0.6
8	205.2	201.5	0.0

[a] Dry weight.

Table 3.—DDT residues on pastures and on hay in the Chenango Valley at various intervals after spray application for gypsy moth eradication near Norwich, New York, 1957.

Farm	DDT Residues in P.P.M. at Days Shown				
	On Pastures[a]				On Hay
	0	7	30	60	120
A	348.0	65.1	6.0	9.6	1.8
B	43.2	37.8	5.4	2.2	7.8
C	147.6	100.8	33.6	17.6	19.8
D	21.6	12.6	7.9	3.0	43.2
E	24.0	21.6	4.1	2.3	1.8

[a] Green weight.

that DDT residues were found present on hay stored in the barns as late as 120 days after application (table 3).

It is readily apparent from the data given in tables 4 and 5 that residues of DDT were present in the milk in detectable amounts up through the last sampling period on all farms surveyed. The amount of DDT residue found in the milk by the method of Mann & Carter (1951) varied between farms and did not appear to be correlated with the DDT residues from pastures. These differences may have been caused by individual management practices or by feeding schedules that differed between farms, or merely by inadequate sampling for a valid estimation of the residues of DDT on the grass.

The dilution of the milk at the milk plants with milk from the untreated areas reduced the level of DDT generally found on the farms but even so was present in chemically detectable amounts throughout the sampling period. DDT was found to be present in the milk up to 1

Table 4.—Residues of DDT in raw milk from farms and milk plants in the Chenango Valley at various intervals after spray application for gypsy moth eradication. Norwich, New York, 1957.

Farm	DDT Residues in Milk in P.P.M. at Days Shown					
	7	30	60	90	210	325
A	0.17	2.90	1.30	0.56	1.10	0.17
B	2.54	3.60	2.20	0.61	1.00	0.20
C	1.42	0.26	0.00	0.00	0.26	0.32
D	3.77	3.50	1.00	0.21	1.20	0.05
E	2.50	2.00	0.66	1.23	2.90	0.40
Milk plants						
1	1.22	2.10	0.78	0.46	0.34	0.26
2	1.10	0.30	0.39	0.45	0.44	0.53

Table 5.—Residues of DDT in raw milk from southeastern New York and Long Island at various intervals after spray application for gypsy moth eradication. 1957.

Farm	DDT Residues in Milk in P.P.M. at Days Shown		
	7	30	180
F	0.62	0.17	0.19
G	0.12	2.30	—
H	—	0.90	—
I	0.25	—	—

year following its application for the eradication of the gypsy moth.

Whole eggs from a commercial poultry farm were analyzed for DDT. One day following spray applications, 0.57 p.p.m. of DDT was present in the eggs while 0.12 p.p.m. was found at the end of 1 month on this same farm. Eggs taken prior to the spraying contained no DDT.

The data would suggest that whatever the attributes of DDT are for gypsy moth eradication it should not be used in dairy farm areas and cannot be so used without contamination of the milk supply.

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The Systemic Insecticidal Properties of Certain Carbamates[1]

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ABSTRACT

Nine carbamates have been investigated as systemic insecticides using the salt-marsh caterpillar (*Estigmene acrea* (Drury)) and lima bean plants as the test system.

Certain of the compounds have proved to be relatively efficient systemic insecticides when applied to the roots under the experimental conditions; others were quite ineffective. On the other hand, with the test system used, no evidence of systemic action has been observed in the young leaves following treatments made to the older leaves. This subject needs further investigation with higher dosages of the insecticides.

Even compounds of very low solubility were effective systemically when applied to the roots. Some of the compounds showed marked phytotoxicity, but others have shown no phytotoxicity at insecticidally effective concentrations.

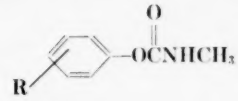
When the behavior of one compound, *m*-isopropyl phenyl *N*-methyl carbamate was investigated, it was found that it accumulated at higher concentrations in the leaves than in the rest of the plant. It was not selectively absorbed or rejected by the roots. Decomposition in the leaves was comparatively rapid.

Certain carbamates are by now well known as insecticides, for example, dimetan, Isolan®, and Sevin®, and they have been shown to be effective against a wide range of insects including aphids, thrips, lepidopterous larvae, granary weevils, and bed bugs. For the most part they have been tested and used as direct contact or stomach poisons, although some have been shown to possess well-developed systemic properties (Gasser 1952).

The studies in the present paper are concerned almost entirely with the systemic properties of the compounds. In the experiments, Isolan and Sevin have been tested together with seven other carbamates, the systemic properties of which do not appear to have been studied previously.

The investigation has two aspects; one deals with the effect of the compounds on the first instar larvae of the salt-marsh caterpillar (*Estigmene acrea* (Drury)) feeding on seedling Henderson bush lima bean plants and the other concerns the level of anticholinesterase activity present in the tissue of the beans treated with one of the most promising of the compounds.

MATERIALS.—The compounds used in this investigation were highly purified by distillation or recrystallization. They included Isolan® (5-(1-isopropyl-3-methyl-pyrazolyl) dimethylcarbamate (b.p. 86-96/0.1 mm.)); Sevin® (1-naphthyl methylcarbamate (m.p. 142–3°)), and the following substituted phenyl *N*-methylcarbamates: *m*-isopropyl- (m.p. 72–3°), (Compound 5727 Hercules Powder Co.); *m-tert*-butyl-(m.p. 144–5°), *m*-dimethylamino (m.p. 86–7°) Kolbezen *et al.* (1954); and *o*-ethoxy-(m.p. 79.5–80.5°), *m*-ethoxy (m.p. 55–60°) *m-n*-butoxy (m.p. 54.5–5.5°), and *m-sec.* butoxy (m.p. 52–4°) (Metcalf *et. al.* 1960). The latter compounds are referred to in the text and tables by the identity of the phenyl substituent R in the general formula:



All the compounds were soluble in acetone, but with the exception of Isolan the solubility in water was low; sometimes very low. The actual values obtained as a result of stirring known weights of the compounds with increasing volumes of water using a magnetic stirer are given in table 1.

The compounds exhibit a very wide range of solubility from less than 0.5 p.p.m. for the *meta-tert*-butyl analogue up to that of Isolan which is completely miscible.

In order to conserve the materials and save labor, it was convenient to store solutions of the compounds; for this reason it was important to know whether they were stable in solution. Warburg estimations of anticholinesterase activity on fresh and stored samples indicated 1% w/v solutions in acetone may be stored in the refrigerator at about 0° C. for several weeks. Aqueous solutions, made by diluting the acetone solutions with water, also stored well at 55° C. for up to 14 days—the maximum tested.

METHODS.—All the tests were carried out in a shaded greenhouse maintained at 25° to 27° C. and about 60% relative humidity. The lima beans (*Phaseolus lunatus*

[1] Paper No. 1221, University of California Citrus Experiment Station, Riverside. Accepted for publication May 9, 1960.

[2] W. A. L. David was on leave from the Agricultural Research Council, Unit of Insect Physiology, Cambridge, England, and in receipt of a Fellowship from the W. K. Kellogg Foundation, Battle Creek, Michigan.

Table 1.—The solubility of the carbamates in distilled water at 30° C.

COMPOUND	SOLUBILITY IN WATER AT 30° C., P.P.M.
Isolan	∞
Sevin	40
R=	
m-iso-C_3H_7	85
m-tert-C_4H_9	<0.5
m-$N(CH_3)_2$	700
o-O C_2H_5	2,300
m-O C_2H_5	1,250
m-n-OC_4H_9	90
m-sec-OC_4H_9	80

(L.)) were sprouted in trays of vermiculite and used for the tests when the first two leaves were well grown (about 2 inches long from tip to petiole) but before the shoot and first trifoliate leaf had begun to elongate.

Ordinarily the roots of the test plants were just washed free of vermiculite and placed in the test solutions contained in 250-ml. beakers covered with a slit filter paper lid.

In the cut-taproot test the main root was cut under water with a sharp razor blade and placed in a shell vial 1 inch long and $\frac{3}{16}$ inch in diameter which held the 1-ml. dose of insecticide solution. The tube was hung to the stem with a cotton thread. The lateral roots of the plant were allowed to take up water from a 250-ml. beaker over which the plant was suspended (David & Gardiner 1951).

If tests were to be carried out with bean plants in soil they were transferred at a younger stage than that described above to tall 7-oz. waxed paper cups which held 205 gms. of moist potting soil. A drainage hole was punched in the side of the cup near the base. The plants were allowed to grow in the soil until they had reached the normal test size. They were then watered with 20 ml. of the insecticide solution and subsequently given controlled amounts of water so that the soil was kept moist, but none drained away.

The biological tests were carried out by placing leaves cut from the treated plants in Munger cells together with two first instar salt-marsh caterpillar larvae. This allowed the larvae access to a disc of leaf 1 inch in diameter (Metcalf & Carlson 1950). The larvae were examined after 1 and 2 days and at the time of the second examination the treated leaves were replaced by leaves from untreated plants. The surviving larvae were allowed to feed on these for 2 days before the final examination. It is the result of this final examination that is given in the tables. At each examination a visual estimate was also made of the percentage of leaf consumed. This provided a further guide to the effect of the treated leaves on the larvae. The experiments were repeated at least five times.

At the time the leaves were cut for the bio-assay, they were examined for symptoms of phytotoxicity which were assessed as follows:

N—leaf normal
T—slight marginal scorch or spotting just detectable
M—Pronounced marginal scorch or spotting
H—More than half the leaf damaged

The insects and test plants were used because they were readily available. The methods were also adapted to use only small amounts of each chemical. In fact, about half a gram of each has been used in all the tests.

The plant material required for the assay of anticholinesterase activity in the tissues was triturated with washed sand and extracted with water. The supernatant fluid was decanted and centrifuged at 1 to 200 r.p.m. and 0.1 ml. was added to a total of 3 ml. of buffer containing house fly (*Musca domestica* L.) head cholinesterase and 0.01 M acetyl choline in the standard manometric assay. The per cent inhibition was converted to micrograms of carbamate by reference to a standard curve (Metcalf & March 1950, Kolbezen *et al.* 1954).

RESULTS OF INSECTICIDAL TESTS.—*Direct Contact and Stomach Poison Action.*—As a preliminary to the work on the systemic properties of the compounds, the salt-marsh caterpillar larvae were fed directly on the treated leaves.

The solutions applied to the leaves were never more concentrated than 0.02% w/v and usually 1 ml. was applied in drops spread evenly over the leaves when they were about 2 inches long. Care was taken to support the leaves on a framework during treatment so that none of the solution ran down onto the roots. The newly hatched larvae were placed on the plants within 2 hours of the leaves' drying or 2 days later. The results obtained after the larvae have been in the Munger cells for 4 days are shown in table 2.

The above results indicate the following order of effectiveness for the compounds tested directly after application: *m-tert*-C_4H_9 > Sevin > *m*-iso-C_3H_7 = *m*-$N(CH_3)_2$ = *m*-OC_2H_5 > Isolan = *o*-OC_2H_5 > *m-n*-OC_4H_9 and *m-sec*-OC_4H_9 which gave no kill.

The last two compounds listed were also the only ones which scorched the leaves within 2 days of application.

Systemic Action Following Absorption by the Roots from Solutions.—Bean plants were placed in 50 ml. of the solutions in beakers as described. Sample leaves were cut for the Munger cells 1 and 3 days later. When the larvae were examined after 4 days in the cells, it was found that there was no significant difference between the kills produced by leaves cut on the first and on the third day. Table 3 gives the results obtained and also shows the phytotoxic effect of the solutions on the plants after 3 days in the solutions.

From the detailed data on which the above results were

Table 2.—Toxic action of the carbamates when first instar salt-marsh caterpillar larvae were fed directly on the treated leaves.

COMPOUND	LARVAE EXPOSED TO LEAVES	
	Within 2 Hours (LD_{50} mg./leaf)	After 2 Days (LD_{50} mg./leaf)
Isolan	0.10	>0.10
Sevin	0.005	0.01
R=		
m-iso-C_3H_7	0.05–0.01	>0.10
m-tert-C_4H_9	<0.005	0.01
m-$N(CH_3)_2$	0.05–0.005	0.10
o-OC_2H_5	0.10	>0.10
m-OC_2H_5	0.05–0.01	>0.10
m-n-OC_4H_9	>0.10	>0.10
m-sec-OC_4H_9	>0.10	>0.10

Table 3.—Toxic action of the carbamates on first instar salt-marsh caterpillar larvae fed on the leaves of bean plants which had absorbed the solutions via the roots.

COMPOUND	CONCENTRATION OF SOLUTION (% w/v) GIVING 50% KILL	PHYTOTOXIC EFFECT ON THIRD DAY CONCENTRATION %/LEVEL
Isolan	0.01 –0.001	0.02/M; 0.01/T
Sevin	0.01 –0.001	0.02/M; 0.01/T
R=		
m-iso-C_3H_7	0.001–0.0001	0.01/T; 0.001/N
m-tert-C_4H_9	0.001–0.0001	0.002/N
m-$N(CH_3)_2$	>0.02	0.02/T; 0.01/N
o-OC_2H_5	0.01 –0.001	0.02/H; 0.01/M
m-OC_2H_5	0.02 –0.01	0.02/H; 0.01/M
m-n-OC_4H_9	>0.02	0.02/N
m-sec-OC_4H_9	>0.02	0.02/N

based, it was concluded that the order of effectiveness of the compounds was: *m-iso*-C_3H_7 = *m-tert*-C_4H_9 > Sevin > Isolan = *o*-OC_2H_5 > *m*-OC_2H_5 > *m*-$N(CH_3)_2$ > *m-n*-OC_4H_9 and *m-sec*-OC_4H_9.

The three most effective materials were also the only ones that did not cause plant injury at insecticidal concentrations. The last two compounds killed no insects at 0.02% w/v, which was the maximum concentration tested.

It was interesting to find that although the *m-tert*-butyl analogue was of very low solubility in water, it was still highly effective as a systemic insecticide.

Systemic Action Following Known Doses Given by the Cut Tap Root.—It has been shown that a known dose of a systemic insecticide can be administered rapidly to a broad bean plant (*Vicia faba* L.) by placing the cut end of the tap root in a small vial containing 1 ml. of the solution while some of the lateral roots are allowed to take up water (David & Gardiner 1951). This method can also be used with lima beans, although it does not work as well since the roots are more brittle and absorption is less certain.

The results obtained by this technique with solutions of the carbamates are shown in table 4. The leaves were cut about 24 hours after the dose had been absorbed.

These results place the more toxic compounds in the same order of effectiveness as was obtained in the experiment on absorption from solutions (table 3).

Table 4.—Toxic action of known dosages of the carbamates on first instar salt-marsh caterpillar larvae fed on the leaves of bean plants dosed via the cut taproots.

COMPOUND	CALCULATED DOSE IN LEAVES (LD_{100} MG./KG.)[a]
Isolan	80–100
Sevin	30– 40
R=	
m-iso-C_3H_7	20– 25
m-tert-C_4H_9	25–30
m-$N(CH_3)_2$	>120
o-OC_2H_5	>120
m-OC_2H_5	>120
m-n-OC_4H_9	>120
m-sec-OC_4H_9	>120

[a] The dosage is expressed as mg. of compound absorbed via the cut taproot per kg. of fresh plant tissue.

Table 5.—Toxic action of the carbamates, applied to the soil in which lima bean plants were growing, on first instar salt-marsh caterpillar larvae fed on the leaves.

COMPOUND	LARVAE FED ON LEAVES ON FOLLOWING DAYS AFTER TREATMENT (LD_{50} mg./pot.)		PHYTOTOXIC EFFECTS ON LEAVES ON DAY 4 AT MAXIMUM CONCENTRATION
	2	4	
Isolan	10–20	>20	N
Sevin	4–10	20	N
R=			
m-iso-C_3H_7	2	2–4	N
m-tert-C_4H_9	2	2	N
m-$N(CH_3)_2$	>20	>20	N
o-OC_2H_5	> 4	> 4	H
m-OC_2H_5	> 4	> 4	M
m-n-OC_4H_9	>20	>20	N
m-sec-OC_4H_9	>20	>20	N

Systemic Action Following Absorption from Soil.—For these experiments bean plants growing in 205 gms. moist soil were used as described under "Methods." The insecticides were applied in a volume of 20 ml. as solutions or, if they were not sufficiently soluble, as fine suspensions from acetone. Sample leaves were cut from the plants 2 and 4 days after treatment. In certain experiments other leaves were cut 7 and 14 days after the treatments to confirm that very slow absorption was not taking place. The kills of larvae with these samples were never higher than those obtained with the samples taken after 2 days. The results are given in table 5. The order of effectiveness was the same as that previously observed with root absorption from solutions.

Systemic Action Following Application to the Older Leaves.—Systemic action on first instar salt-marsh caterpillar larvae feeding on the first trifoliate bean leaf has been investigated following application of the compounds to the first pair of leaves. In the case of the *m-tert*-butyl compound, 3 ml. of an 0.002% solution were applied, with Sevin 3 ml. of an 0.01% solution, these concentrations being in each case near the maximum which could be prepared by diluting 1.0% w/v solutions of the compounds in acetone with water. For all the other compounds 2 ml. of an 0.02% solution were applied representing a dose of 0.4 mg. on the plants.

At the maximum dosages tested, none of the compounds affected larvae fed on the young untreated leaves and the *m-n*-OC_4H_9 and *m-sec*-OC_4H_9 isomers both caused moderate amounts of damage to the treated leaves. Higher dosages were not evaluated because with the formulations employed, the necessary concentrations of acetone would have been objectionable.

The *m*-isopropyl analogue was selected because it was both toxic and reasonably soluble in water. Using the Warburg technique to measure anticholinesterase activity a standard curve was first determined for the compound in the presence of bean tissue.

Distribution of the m-isopropyl Analogue Between the Roots, Stems and Leaves of Bean Plants Absorbing the Solution via the Roots.—Four beakers were prepared each containing 50 ml. of 0.001% w/v solution of the analogue

Table 6.—The distribution of the *m*-isopropyl analogue between the roots, stems, and leaves of bean plants absorbing the solution via the roots.

Plant Assayed After Hours in Solution	Concentration Found (μg./gm.) in:		
	Root	Stem	Leaves
First Experiment			
7	15	30	46
28	24	26	61
48	23	32	52
120	20	32	87
Second Experiment			
24	13	12	14
48	29	32	52
72	24	32	64
120	21	31	70

to which more solution was added later as it was absorbed. Four bean plants were placed in each beaker and the plants were removed after the intervals shown in table 6. At the time of removal, the roots and lower parts of the stems were washed thoroughly in running water. They were then dried and separated into roots, stems, and leaves for assay.

From table 6 it can be seen that the concentration of active material in the root and stem seems to reach a more or less steady maximum while that in the leaves continues to increase. The concentration in the leaves is also higher than that in the other parts of the plants; in this respect the behavior of the analogue resembles demeton-thiol isomer and differs from schradan and dimefox (David 1951, 1952, 1957).

Selective Absorption or Rejection of the Analogue by the Roots of Bean Plants Absorbing the Solution.—It has been shown previously that the roots of broad bean plants selectively reject schradan and selectively absorb dimefox when allowed to take up the solutions over several days (David 1951, 1952). Experiments were therefore set up to determine whether the *m*-isopropyl analogue was selectively absorbed or rejected by the roots of lima bean plants.

By the time that up to an average of 52% of the solutions had been absorbed in six experiments, no change in the concentration of the 0.001% solution used was detected when this was compared with control solutions not containing plants. It may be concluded that the solution was absorbed unchanged.

Persistence of the m-isopropyl Analogue in the Leaves of Plants Treated Systemically via the Roots.—The persistence of anticholinesterase activity, calculated as the *m*-isopropyl analogue, present in the leaves of treated plants has been determined (a) in the case of plants removed from the solution and placed in water (b) in the case of leaves cut from the plants and stored in Munger cells as was done in the insecticidal tests.

When the samples of leaves were cut for placing in the Munger cells, all were weighed and some were immediately assayed for anticholinesterase activity. The value obtained indicated a concentration of 125 μg. of the *m*-isopropyl analogue per gram of fresh leaf tissue. This value fell rapidly in the leaves in the Munger cells and even more rapidly in those left on the plants transferred to water. (See table 7).

Table 7.—Persistence of the *m*-isopropyl analogue in the leaves of plants treated systemically via the roots.

Time After Cutting Leaves or Removing Plants from Solution (hours)	Concentration Found (μg./gm.)[a] in:	
	Leaves Stored in Munger Cells	Leaves Left on Plants
24	104	78
28	32	24
72	31	22

[a] Micrograms of *m*-isopropyl analogue per gm. of fresh leaf tissue.

Discussion.—The carbamates tested showed a wide range of toxicity to first instar salt-marsh caterpillar larvae as judged by the results obtained when they fed directly on the treated leaves. The most effective killed the larvae at a dosage of less than 0.005 mg. per leaf of some 3 sq. inches of area which is equivalent to a dose of less than 0.4 oz./acre of leaf surface. At this dosage the larvae ate only a minute amount of the 1-inch diameter leaf disc available to them. All these considerations suggest that in practice, a crop of leaf area 10 to 20 times that of the land on which it is growing, would require less than 8 oz./acre for effective control.

In the experiments conducted, the carbamates which were most effective as contact-stomach poisons were also the most effective systemically when applied to the soil. Unfortunately, it is much more difficult to translate the results of systemic insecticidal tests carried out in small plots to terms of field application. A comparison may be made, however, with other insecticides tested in essentially the same way. Thus, the *m*-isopropyl and *m-tert*-butyl analogues killed 100% of salt-marsh caterpillar larvae at a dosage of about 2 mg./200 gm. of soil. Demeton applied in the same way killed *Aphis fabae* Scop. on broad beans at 1.0 mg./400 gm. soil and newly hatched *Pieris brassicae* L. larvae at about 40 mg./400 gm. soil.

Determinations made by the cut-taproot technique showed that the *m*-isopropyl and *m-tert*-butyl analogues gave 100% kills of the salt-marsh caterpillar larvae at between 20 and 30 mg. per kilogram of fresh leaf tissue. Demeton killed all *Aphis fabae* Scop. at about 1 mg. per kilogram of fresh plant tissue and would have killed third instar *Pieris* larvae at between 140 and 310 mg. per kilogram of fresh plant tissue. It may be concluded that some carbamates definitely show promise as systemic insecticides. It is interesting to note that despite its very low solubility of less than 0.5 p.p.m., the *m-tert*-butyl analogue was one of the two most effective compounds.

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Artificial Bee Beds for the Propagation of the Alkali Bee, *Nomia melanderi*[1]

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ABSTRACT

The construction of artificial beds for the propagation of the alkali bee (*Nomia melanderi* Ckll.) was achieved by duplicating the texture, moisture, and alkalinity of the native soils in which the bee was found. Pits, up to 60 by 60 feet in outside dimension, were excavated 3 feet deep and lined with polyethylene. Two to four inches of gravel were added to the polyethylene and the pits backfilled with soil consisting of less than 7% clay-size particles. Salt was rotovated into the upper level of the bed to effect soil dispersion and reduced water loss. Tubes were led through the soil to the gravel layer as a method of introducing the necessary moisture, and transplants were made into the bed to serve as the nucleus of the new colony. Burrowing populations in excess of 250 per square foot were recorded from a 2-year-old bed, which represents an 8-fold increase in population density over the best natural site in Oregon.

As reported in previous papers (Stephen 1959, Bohart *et al.* 1960, Stephen & Evans 1960) the alkali bee is the principal reason for the high seed yields in alfalfa in parts of western America. The present studies were initiated to investigate the ecology of the alkali bee, and to determine the factors that appear to restrict its expansion. In any given species the factors contributing to a restriction of the population size are numerous. Regardless of their number, however, there is always a single factor that will exert the most strigent limitation on population increase. In overcoming this limitation a step towards a more complete realization of the reproductive potential will have been made. It has been the author's opinion that the increase in the intensity of cultivation plus the extensive drainage and reclamation programs that have been conducted in the areas to which the alkali bee is endemic have seriously restricted the availability of bee nesting areas. The absence of optimal nesting areas has therefore been considered the principal limiting factor to population increase.

The factors found to be common to all excellent natural nesting sites on which analyses have been made appears to be a low clay-size particle content plus high moisture at the surface of the nesting site. Soils with an aggregated clay-size particle content of less than 8% permit a rapid capillary transport of subsoil water, and when soils of this composition are found in association with a high water table, conditions for alkali bee inhabitation have been partially met (Stephen & Evans 1960). The most critical factor determining the population concentration appears to be the presence of sufficient permanent moisture at the soil surface. The amount of moisture necessary to maintain this condition is a relative matter which depends on the retaining capacity of the soil. Highly aggregated clay soils will require more than 20% water while lighter soils, low in aggregates, will yield optimal working surfaces at a 10% level.

Under natural conditions the greatest concentration of alkali bees was always found in association with a compact or deflocculated soil surface. Many sites were accepted by alkali bees during the spring when the soil surface was moist but were abandoned as the surface 2 inches began to dry out or as the surface became increasingly flocculated or fluffy.

Investigations on the effect of salts on nesting soils has shown that the necessary deflocculation and compactness of the soil surface can be achieved through the addition of monovalent salts such as sodium or potassium. The presence of excessive sodium also appears to convey a water retaining capacity to the soil, and influences the degree of divalent cation solubility in those soils showing flocculation or salt precipitation (Stephen 1960b, 1960a).

Since large populations of the alkali bee are found principally in areas of slow seepage, they are often removed by some distance from alfalfa fields being retained for seed production. In many other locales, soil conditions considered adequate for alkali bee inhabitation cannot be found in areas where seed production is being contemplated. It has been necessary to raise alfalfa seed in areas where the bee abounds.

This paper is a report on the attempts to create artificial nesting conditions in proximity to alfalfa fields being retained for seed, and the subsequent establishment of sufficiently large-size colonies to make the production of alfalfa seed economically practical.

[1] Supported in part by a grant from the Malheur Seed Growers Association. Technical Paper No. 1314, Oregon Agricultural Experiment Station. Accepted for publication May 16, 1960.

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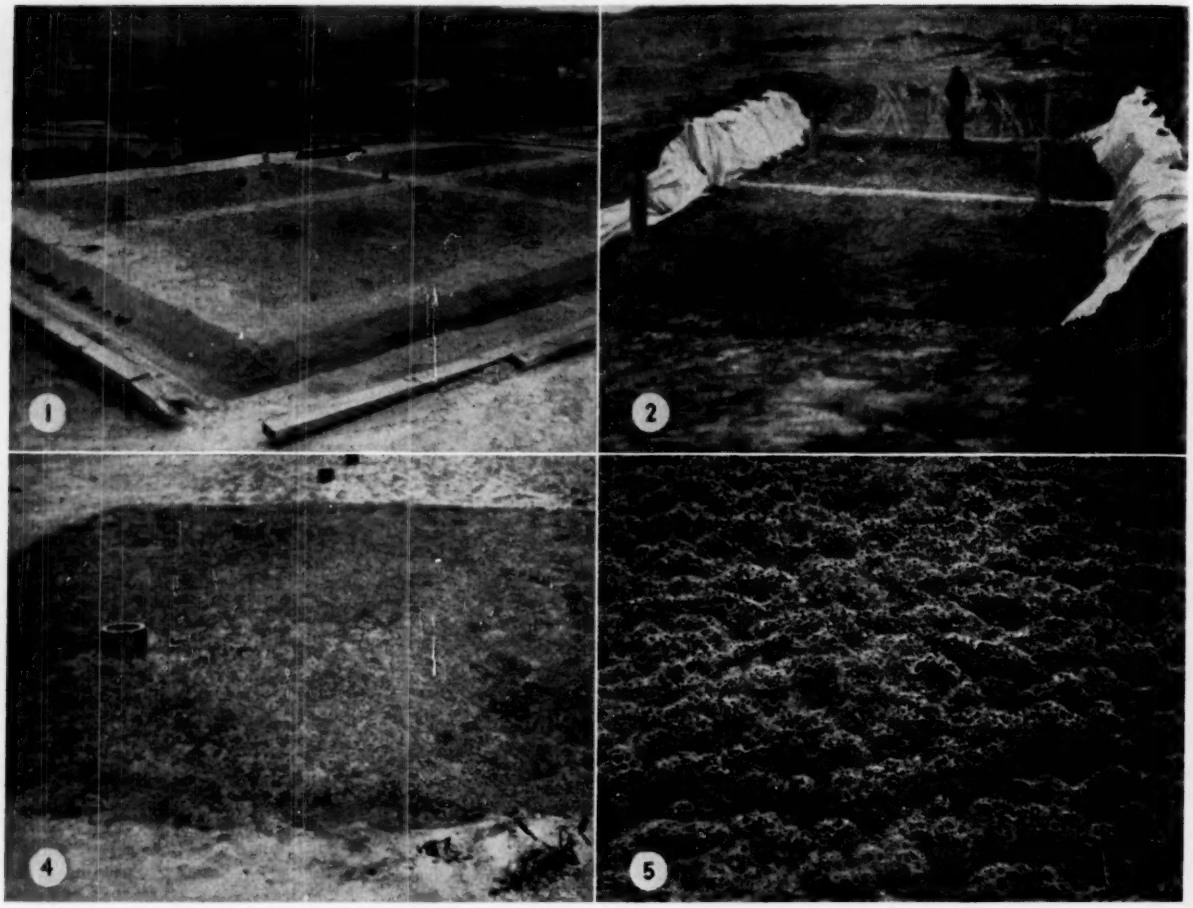

FIG. 1.—Four 8×12-foot artificial beds showing tops of water downspouts in each bed. The roughened areas indicate the extent of bee occupancy during the first year.
FIG. 2.—A 60×30-foot artificial bee bed in the early stages of construction. The polyethylene is covered with 4 inches of gravel to serve as a method of rapid lateral water transport.
FIG. 4.—A 2-week-old artificial bee bed in the foreground indicates the restriction of burrowing activity within the confines of the polyethylene.
FIG. 5.—View of the surface burrowing in a 2-year-old artificial bee bed.

METHODS.—The first attempts to create an artificial nesting site for the alkali bee were made in 1957 employing a horse watering-trough as the bed. Several inches of gravel were placed in the bottom of the trough which was then filled with soil having clay-size particle content of 5% on aggregate analysis. A concrete pipe was led down to the gravel layer and the water added through the pipe so that it could percolate up through the soil and maintain a uniform surface moisture. Transplants from existing alkali bee beds were made into the trough while adult activity was in progress. This was accomplished by driving an open end 5-gallon can into the soil at an active nesting site, and removing the can with its contents to the site of the new bed. Transplanting in the early morning hours, prior to the time of bee emergence, resulted in the successful transport of entire burrows containing immatures as well as adult females. The cans with their contents were set directly into the soil. In other instances, the can was removed and only the soil core placed in sites.

Early in 1958, three other types of beds were constructed. The first type consisted of wooden boxes, 4×8 feet in dimension and approximately 30 inches high. Each box was lined with 6 mil polyethylene, to insure against water loss. Gravel, soil with a low clay-size particle content, and access pipes were added as outlined for the beds above. Sodium in the form of sodium chloride was added to the backfilled soil at rates of 10 to 100 pounds per bed. In some beds it was mixed throughout the backfilled soil, while in others it was added only to the upper 18, 12 or 6 inches. The soils were wetted and transplants containing overwintering prepupae were introduced to each bed just prior to emergence.

Other types of beds, constructed the same spring, were designed to be permanent installations in close proximity to fields being retained for alfalfa seed production. In these beds the soil was excavated to a depth of 24 to 36 inches and construction proceeded as outlined above. A series of four small beds, each 8×12 feet were dug 24 inches deep. Each pit was lined with 6 mil clear polyethylene and 2 to 4 inches of gravel placed over it. Soil with an aggregated clay-size particle content of less than 6% was backfilled and compacted. Two clay pipes, 3 inches in

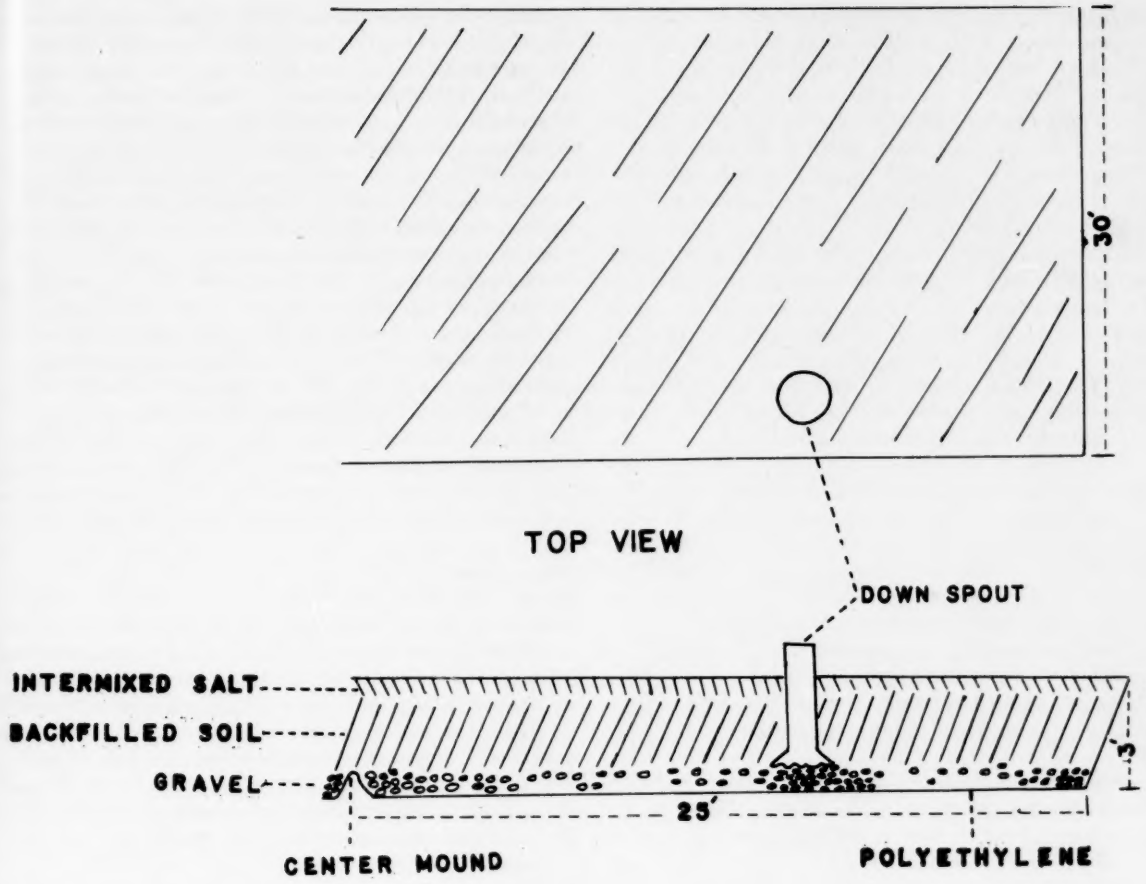

FIG. 3.—Sketch of a large artificial bee bed in cross-section.

diameter were led through the soil to the gravel as a means of supplying the necessary water to each bed (fig. 1).

Since larger bee beds appeared to be more practical, the size of beds was increased in 1958 and 1959. The dimensions were determined by the largest available sheets of polyethylene. Each bed 30×50 feet was excavated to a depth of 30 to 36 inches and lined with 6 mil polyethylene.[3] Construction proceeded as outlined above, except for the construction of a 12-inch mound of soil across the middle of the bed, prior to the addition of the polyethylene (fig. 2). This resulted in an interruption of the otherwise continuous gravel layer, so that if a severe rupture in the water-retaining polyethylene occurred, the entire bed would not have to be abandoned owing to an inability to supply sufficient moisture. From 1,000 to 3,000 pounds of salt[4] were intermixed with the backfilled soil, or applied to the surface of the completed bed and rotovated in to a depth of 2 to 4 inches. The effect of the addition of salt to the various levels of the soil is reported in a previous paper (Stephen 1960a). A single concrete pipe, from 3 to 8 inches in diameter extended from the soil surface to a small mound of gravel, in each half of the bed. The gravel was mounded to permit a more rapid transportation of water away from the base of the pipe and insure a more uniform vertical capillarity through the backfilled soil (fig. 3).

After the bed had been completed, sufficient water was added through the downspouts to bring the moisture level of the entire backfilled soil up to a level optimal for bee activity. As indicated in an earlier paper, the amount of moisture necessary to achieve an optimal level varies with the texture of the soil (Stephen & Evans 1960). Soils high in clay- and small silt-size particles held in the form of larger aggregates will require considerably more water than an unaggregated sandy-silt.

To determine the amount of water necessary in each bed, the per cent moisture of the backfilled soil was first obtained. Then, by calculation based on the volume of soil in each bed, it was possible to arrive at the approximate quantities of water necessary to elevate the moisture content to the 12%, 14% or 24% demanded of the different textured soils. This ranged from approximately 2,000 gallons in the wetter, lighter soils to 9,000 gallons in the

[3] The polyethylene was donated to this project by the Visking Corporation, Chicago, Illinois.

[4] No. 5 Hay and Stock Salt used in these experiments was donated by Leslie Salt Co., San Francisco, California, and Salt Lake City, Utah.

drier, highly aggregated soils. Once the moisture appeared at the soil surface it was found desirable to recompact the surface by driving a vehicle back and forth over the bed.

Ten to twenty soil cores containing prepupae of the bees were then transplanted into each artificial bed, using a transplanting device developed by the author. The transplants were made in the spring shortly before emergence. It was estimated that each core contained approximately 200 prepupae.

RESULTS AND DISCUSSION.—The small artificial beds constructed in 1957 using the horse troughs indicated that it was feasible and practical to establish bees. The transplants were made during the middle of the active flight season by sealing the holes and moving the soil cores intact. The females quickly became oriented to the new surroundings and proceeded to work in a normal manner. Great difficulty was experienced in trying to remove the soil cores from the 5-gallon containers. Each attempt resulted in a greater or lesser degree of fragmentation and resulting loss of bees. The several samples that were set into the new bed with the can still enclosing them fared very well at first. However, within 2 weeks the surface of the soil within the cores began to dry and become powdery, even though the surface about it remained relatively moist and compact. It was assumed that presence of the can prohibited the lateral transmission of moisture from the surrounding soils, a factor which made the transplanting method impractical. Sufficient numbers of bees survived the first winter, and the following spring they began to extend into the soils of the trough. It was evident from these trials that the principal restricting factor to the maintenance of alkali bees in artificial sites was the absence of sufficient soil moisture particularly in the upper levels of the beds.

The twelve 4- ×8-foot boxes were constructed in an area considered free of native alkali bees, or else so few in number that they were not found even after prolonged searching. Of the estimated 200 prepupae in each of the cores transplanted into each bed there resulted a "take" of from 35 to 60 nesting females. Considering that some mortality was experienced in emergence from the soil, and half of the pupae were males, this would represent from 35% to 60% success. Under field conditions, an average of 53 females was found to emerge from equivalent areas in the bed from which the cores were taken. This would indicate that this method of transferring bees could be considered highly successful. In these box beds there was a direct relationship between the amount and placement of the salt and the appearance of an optimal soil surface. Observations indicated that the higher salt levels (over 1 pound per square foot) gave the most optimum surface conditions when compared with the better natural sites.

In the series of permanent 8- ×12-foot beds, the bees began emerging about 2 weeks after the transplants were made. It was assumed that these beds were being constructed in an area that was free of alkali bees since, except for a single 25-acre alfalfa field being kept for hay, there was no alfalfa within several miles. Once emergence began in the artificial sites, alkali bees began nesting in close proximity to the transplants but, within a week, the population had spread over the entire bed. This increase continued for a period of 3 weeks, at which time the population of the beds reached approximately 90 burrowing females per square foot. Two similar-sized beds were quickly constructed immediately adjacent to these beds and they too were populated to the same extent before the summer ended. From this, as well as other similar experiences, it was concluded that the emerging bees serve as an attractant to other bees in the area. Apparently there was a scattered residual population of the bee in most of the region of endemism, and these individuals barely maintain themselves under submarginal conditions. The sudden appearance of optimal nesting conditions in association with an established nucleus of bees serves as a strong attractant to newly emerged females seeking a promising site in which to burrow. It is interesting to note in these sites that, even though the population during the first season was well more than three times as dense as any seen under natural conditions, they restricted their burrowing activity sharply within the confines of the polyethylene-enclosed soil (fig. 4). The surface of the beds was raised approximately 2 inches by the soil excavated by the bees in the process of burrowing construction. Shortly after emergence began, it was noted that the soils of these beds lost moisture rapidly and the surface tended to become increasingly powdery. Water was added to the downspouts every other day to maintain the surface in an optimal working state. Immediate efforts were made to correct the rate of water loss and to effect a deflocculated surface through the incorporation of salt into the soil. The surface of the beds was brined with a surface application of a 15% salt solution, and each bed was subirrigated with a salt solution of approximately the same strength. Within 2 weeks the surface was highly deflocculated and compact in spite of the dense population of bees, and further addition of water was unnecessary that season.

Several more beds ranging in size from 20×25 feet to 60×60 feet were constructed in the immediate area in early 1959, to hold the overflow from the 1958 populations. The first large, permanent bed, which was approximately 30×60 feet in outside dimension, was constructed in the spring of 1958. This bed was backfilled with soil having less than 5% clay-size particles and had 1,500 pounds of salt intermixed into the upper 18 inches. Sufficient water was added to bring the moisture, and with it much of the dissolved salt, to the surface. It required from 2 to 4 days for the moisture added to the gravel layer to reach the soil surface through capillarity. The bees from the transplants began emerging in mid-June and the earlier emergents burrowed back into the core from which they had come. As the number of emergents increased they began to burrow in the soil adjacent to the transplanted cores, and within a month there was a sparse distribution of bees throughout the bed. During the first month attempts were made to count the numbers of new burrows, but within a week after emergence began, it became impractical. By the end of the summer there was an average of 10 to 15 burrowing females per square foot in the bed, the majority of which had undoubtedly come from other sites located a mile distant. The soil retained a a suitable compact working surface throughout the season with the single application of water made in the early spring. This is attributed to the high monovalent salt concentration at the soil surface which is believed to restrict evaporation or act in a hygroscopic capacity (Stephen 1960b).

Additional water was added to this bed in the spring of 1959 just as the bees began emerging. Under the influence

of lower soil moisture conditions, the bees emerged well before those in the natural beds in the area. This earlier activity appeared to attract many emergents from the natural sites and by the beginning of July the bed was perforated by nesting bees. Ten surface counts were made in early August and the number of burrows ranged from 211 to 276 per square foot (fig. 5). The surface of the soil on the bed had been raised 4 inches by the diggings and larvae were found continuously from 2 inches to 21 inches deep. Figure 6a shows the prepupal population of a core taken from the artificial bed, with a population of 1,809 prepupae per square foot. Figure 6b illustrates a core taken from one of the excellent natural nesting sites with typical layering of the brood at about the 6- and 10-inch levels. In all of the excellent natural bee beds examined during the last 5 years, (characterized by the presence of from 20 to 30 burrowing females per square foot) cores yielded an average of 277 prepupae with a maximum of 254 prepupae in the square-foot samples.

A total of 17 large permanent beds were constructed in 1958 and 1959 and each of these was occupied to a greater or lesser extent during the first year. It was found that the total bee population at the end of the first year could be correlated directly with the number of bees introduced or with the available bee population in the area in which the beds were located. Those beds lying in areas in which the endemic population was low or absent depended on the residual introduced population for expansion.

Under optimal soil conditions and available forage each female will produce an average 18 to 20 cells, half of which will be females. However, the emergence studies in the field and laboratory, as well as the population counts made in the transplants cited above, indicate that a 4- to 5-fold population increase per year can be anticipated under normal conditions.

CONCLUSION.—The larger bee beds appeared to be the most practical of the types of bee beds that have been constructed. With care in the selection of soil and the incorporation of a deflocculating salt in the upper few inches of the bed, the sites can be maintained with minimal effort. The restriction of the dispersing sodium chloride to the upper few inches of the bed permits a rapid rise of water added through the downspouts, making it possible to change the surface moisture content of the bed within 24 hours. In addition the high monovalent salt concentration at and near the surface imparts a strong moisture retention quality to these soils, so that a single application of water in the early spring is sufficient to maintain an optimal working surface throughout the year.

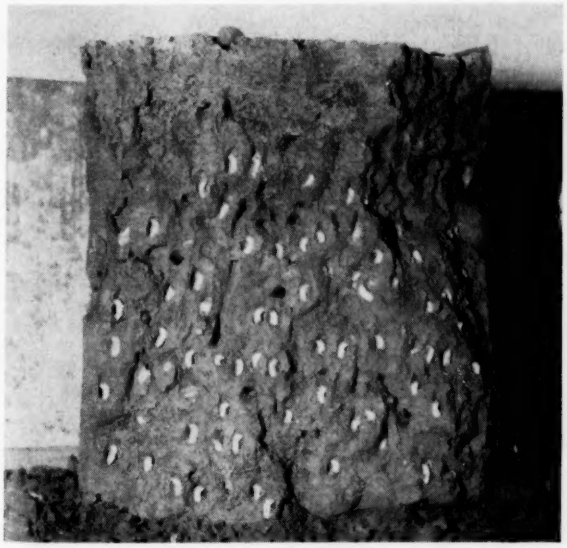

FIG. 6a.—Cross-section of a core taken from a 2-year-old artificial bee bed showing the continuous distribution of prepupae from 2 to 21 inches below the original soil surface.

FIG. 6b.—Section made in an excellent natural bee bed showing the typical tendency of the bees to form two layers of brood when crowded.

The abnormally high populations realized in the artificial sites are attributed to at least two factors. First, the presence of a hardpan, or changes in the soil texture profile, are eliminated by providing a deep soil of uniform texture. The females are able to penetrate to greater depths than under most field conditions. Secondly, the depth of the soil and the single subirrigation in the spring, presents a more uniform soil moisture content throughout the bed. Moisture determinations run on several beds during 1959 indicated that there was no more than a 2% difference between samples taken from the surface 6 inches and those taken from the 24- to 30-inch level. This fact contrasts strongly with field conditions where waterlogged soils are often found from 10 to 16 inches below the surface. This excessive moisture is reflected by the burrowing activities of the bee, which rarely if ever penetrates the wetter soils.

The efficiency of each burrowing female, however, is curtailed where the populations reach those cited previously. With an average of 221 burrowing females per

square foot, there were only 1,809 prepupae in the equivalent area. This would mean that each female is producing slightly more than eight progeny, considerably less than the 18 to 20 average under ideal conditions. This sharp reduction in individual efficiency may be accounted for by depth to which many of the females must dig (up to 20 inches compared with 6 to 10 inches under natural conditions) as well as by the fact that the entrances to each of the burrows are so close, that excavation diggings from one burrow are deposited into the entrances of the adjacent burrows. The habits of the females in keeping the entrances clear at all times consumes much of the time normally devoted to pollen collection and cell construction in these densely populated beds.

It will be interesting to note what effect the overpopulation will have on the behavior of the emergents in 1960, and what the long-term effect of the dense population will have on the acceptability of the bed.

Artificial beds for the propagation of the alkali bee are devised primarily for those areas in which alfalfa seed is being produced or being contemplated, and in which no natural or potential natural sites are available for inhabitation. The artificial bed permits the seed producer to place bees precisely where they will benefit him the most in the production of alfalfa seed. The number of such bee beds that would be required for an economically practical production of seed on an acre basis depends on a variety of factors. Conditions that would govern effective bee utility include: the dilution of the existing bee population by a great excess of available alfalfa being retained for seed; the time of bee emergence in relation to the time of peak bloom; the effectiveness of insect control programs; and cultural management of alfalfa being retained for seed.

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A Wide-Range Variable Temperature Cabinet for Bio-Ecological Studies[1]

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ABSTRACT

A brief description is given of the construction and performance of a variable temperature cabinet. The cabinet offers outstanding program reproducibility and a broad temperature range at low cost.

The need for a device which can provide a controlled variable temperature has been voiced by many workers, and several types of "climate" cabinets have been constructed. None, except very expensive commercial types, would have filled our needs. An inexpensive cabinet capable of working at temperatures ranging from below freezing to well above 100° F. was required in this laboratory to aid in evaluation of the results of artificial selection on several climate-tolerant strains of the California red scale parasite, *Aphytis lingnanensis* Comp. (Hymenoptera: Chalcidoidea: Aphelinidae). Development of a cabinet providing programed temperatures over a wide range was undertaken to fill this need, and, though its construction involves no parts which are basically new, a report of the assembling of readily available components into an inexpensive but very practical "climate" cabinet appears both justified and desirable in view of the fact that all reported climate-control cabinets, except the bioclimatic cabinets (Flitters & Messenger 1953, 1956) and certain commercial types, have operated at temperatures above room temperature.

One major problem has been the development of simple but accurate mechanical controls capable of activating a thermoregulator as needed to give the desired variable temperature program. The method of Stone (1939) and Munger (1944) was not used for two reasons: (1) the use of a thermograph would have appreciably increased the cost of the equipment, and (2) no attempt to simulate a long-range weather cycle was planned because for testing purposes it was considered better to reproduce daily an "ideal" temperature representing a typical summer day or winter day or other idealized weather condition. Such a system necessitates only a single cam which repeats identical cycles daily through one or more generations in testing an insect culture. The mechanical linkage of Howe (1956) appeared to be somewhat difficult to regulate, and a given program might be difficult to repeat following a program change. Finally, the size and cost of the bioclimatic cabinets developed and built cooperatively by the University of California and the U. S. Department of Agriculture (Flitters & Messenger 1953, 1956) place them and other commercial types beyond the scope and means of most laboratories. By elimination, the cam-operated thermoregulator used by Wishart (1940) and commonly

[1] Accepted for publication May 16, 1960.

[2] The authors gratefully acknowledge the assistance rendered by Robert Orth, Richard Morrison, and Eric Halfhill in the construction of the cabinets with which this paper is concerned. They are also grateful to Mr. Orth for the art work involved in preparing the figures.

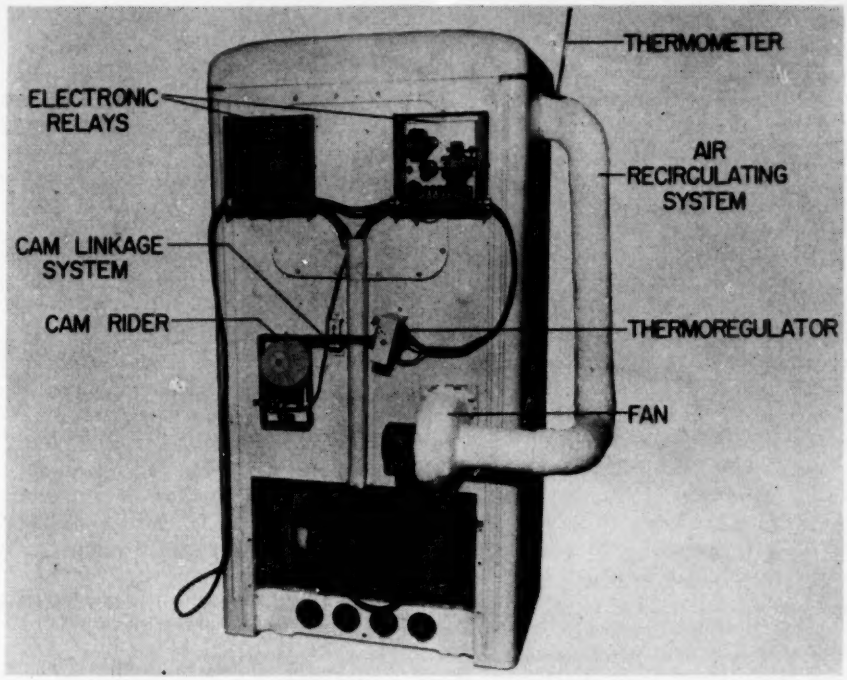

FIG. 1.—Rear of the variable temperature cabinet showing the external conversion details.

employed in commercial equipment appeared most practical and adaptable to a broad spectrum of studies in insect ecology and physiology. Such a system has the characteristic advantage of complete program reproducibility.

CONSTRUCTION.—Since temperatures below freezing as well as above 100° F. were to be required of the device, a household refrigerator was the obvious choice for the basic cabinet unit. Major modifications were required, however, as follows:

(1) The installation of an external air recirculating system (fig. 1). This consists of a small squirrel-cage fan and appropriate insulated duct work which recirculates the air through the cabinet at the rate of 60 cubic feet per minute.

(2) Installation within the cabinet of a 200-watt cone-type heating element (fig. 2(1)). This unit should be installed directly in front of the air current from the fan.

(3) Replacement of the refrigerator's thermostat with a "Quickset" bimetal thermoregulator made by the American Instrument Company, Silver Spring, Maryland (figs. 1 and 3(7)). This thermoregulator was found to fill our needs best. It is equipped with a single-pole, double-throw contacts, has a very wide temperature control range, and is adjusted by rotating the head of the control. Use of this control necessitated the addition of suitable control relays (fig. 1). Very sensitive electronic relays, also marketed by the American Instrument Company, were utilized.

(4) The mounting of a clock-driven cam and linkage system (figs. 1 and 3) capable of rotating the head of the thermoregulator through a measured arc, and thereby adjusting the cabinet temperature to the level dictated by the program cam.

Briefly stated, the ability of the cabinet to reproduce an alternating temperature depends on the single-pole, double-throw characteristic of the thermoregulator which permits either the heater or the refrigeration unit to operate on command of the program cam. The radius of the clock-driven cam acting through the linkage controls the temperature within the cabinet by closing the appro-

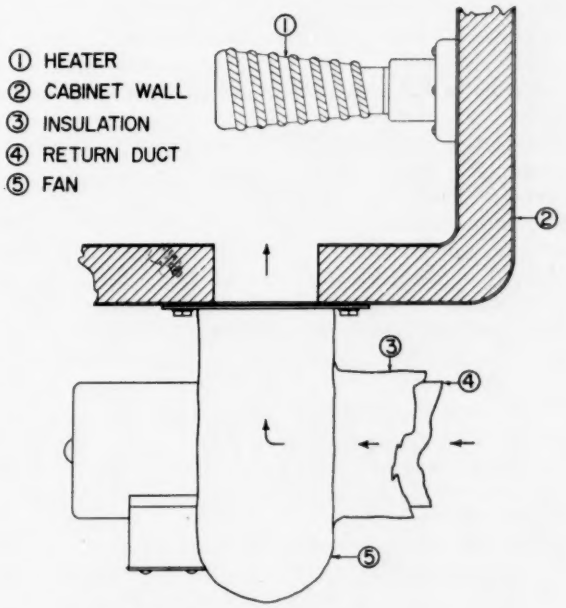

FIG. 2.—Diagrammatic sketch showing fan and heater relationship.

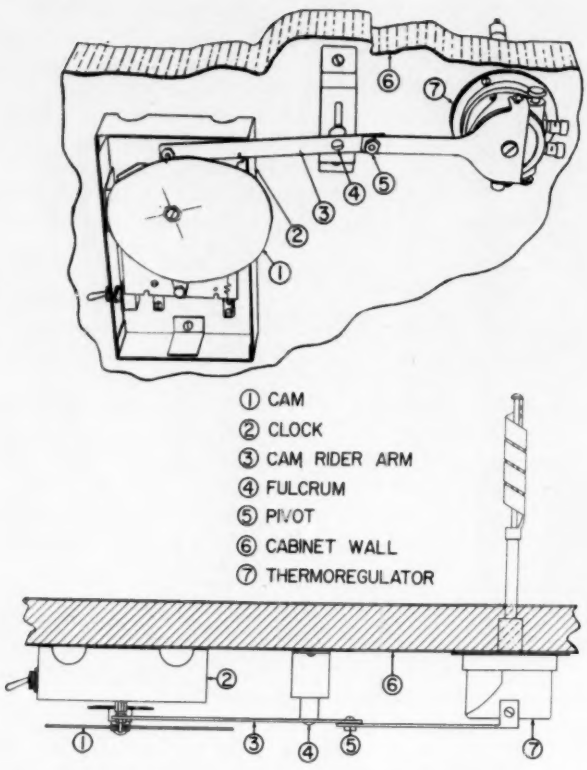

FIG. 3.—The thermoregulator and control mechanism.
Above: rear view.
Below: bird's eye view.

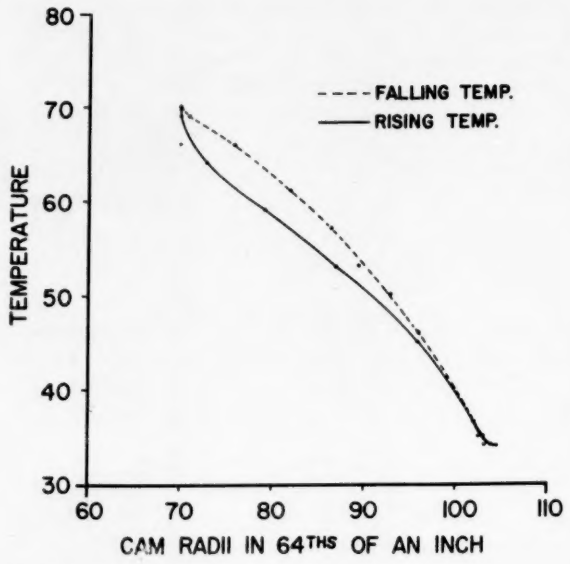

FIG. 4.—A typical calibration graph showing the relationship of temperature to cam radius.

priate contact in the thermoregulator, thus causing either the refrigeration unit or the heater to operate. Accurate setting of the two fine adjustments on the thermoregulator spaces the two outside contacts of the single-pole, double-throw arrangement in such fashion as to permit a lag period between operation of the heater and the refrigeration unit. This lag, as pointed out by other workers, is necessary to the smooth reproduction of a temperature curve. Without it, the heater would turn on as soon as the refrigeration unit turned off and vice versa. The resulting temperature curve could be extensively serrated in appearance. Proper adjustment is approximated when, with the thermoregulator at rest (acclimated), each of the outer poles is spaced about $\frac{1}{64}''$ from the center pole. When properly adjusted, the refrigeration unit will not turn on at all during periods of increasing temperature, and the heater will not operate during periods of decreasing temperature. By providing a cam with a constant radius (*i.e.*, perfectly circular) or by stopping the clock, the cabinet can be made to function at a constant temperature anywhere within its temperature range.

Calibration of the cabinet is not simple. The temperature produced at a given point on the radius of the cam depends on how rapidly the temperature is rising or falling at the time and, to some extent, on how high or how low the temperature has been at its most recent extreme. Therefore, the best means of illustrating the performance of a given cabinet of this type is to prepare a series of calibration charts covering the desired temperature range. Figure 4 shows one such graph. This series, if complete, could consist of a large number of charts, but as a practical matter, a comparative few will normally serve adequately in view of the fact that a cabinet is normally expected to reproduce only a limited number of "climates" in a given series of tests.

For example, if it were desired to calibrate a newly constructed cabinet for the purpose of duplicating a summer cycle and a winter cycle the following steps would be taken.

(1) *Pilot* cam #1 would be prepared for the purpose of measuring the extreme temperatures dictated by a cam with radii ranging from maximum to minimum. These maximum and minimum radii would be determined by the mechanical construction of the cabinet control assembly. The thermograph record of cabinet performance using this cam when plotted on a calibration chart would yield the approximate temperature range of the cabinet and would show the approximate cam level (radii length) required to produce the two programs desired.

(2) *Pilot* cams A and B (representing the summer cycle and winter cycle, respectively) would then be prepared by utilizing the data provided by *pilot* cam #1. These cams would yield performance data within the limited temperature ranges involved in each of the climate cycles.

(3) Preparation of cams A and B (representing the summer cycle and winter cycle, respectively) can then be accomplished by utilizing the refined data provided by *pilot* cams A and B.

This method, though somewhat cumbersome, is not prohibitively laborious. Preparation of a given cam may require from a few minutes to an hour, but time is required for the accumulation of performance data.

Step (3) above is required only when exact duplication of a predetermined climate cycle is required. *Pilot* cams A and B, prepared in step (2), will operate the cabinet control mechanism following the planned program within rather broad limits. When a precise program is demanded, the refinement outlined in step (3) is required.

Cams may be made of a number of materials which are

readily available. Both tempered masonite and $\frac{1}{16}$" sheet aluminum have been used successfully on cabinets constructed in this laboratory. In preparing a cam, the desired temperature for each hour of the day is converted to a radius by using an appropriate graph similar to the one in figure 4. These radii are plotted on a cam blank, the points are joined by a smooth curve, and the resulting cam is cut out with the aid of a jig saw. This rough cam must then be filed carefully to conform to the plotted cam.

PERFORMANCE.—Cabinet temperatures, utilizing a household refrigerator, can be made to range from a minimum of 24° to a maximum well above 125° F. with an accuracy of ± 1° F. However, as the minimum temperature is approached, the rate of decrease declines so that it requires a considerable amount of time to attain temperatures below 30° F. In tests measuring the rate of maximum decline, the temperature fell from 102° to 30° F. in $2\frac{1}{2}$ hours and from 72° to 30° F. in about 2 hours, but in both cases another $2\frac{1}{2}$ hours was required to reach 24° F. This does not necessarily restrict the lower limit of the useful range since, in duplicating a southern California cold-night situation, a smooth decline in temperature to 24° F. can be duplicated. However, because of the slow response below 30° F., the cabinet cannot duplicate sudden temperature declines in this portion of its range.

Maximum temperatures are restricted by the temperature tolerance of living organisms rather than by any mechanical consideration. *Aphytis* species (our test insects) can tolerate temperatures only slightly above 100° F. for very brief periods of time. The maximum rate of increase is limited by the greatest curvature of the cam which can be climbed by the cam rider (figs. 1 and 3) on the thermoregulator control linkage arm. In measuring the maximum rate of temperature increase (without using the cam), a rise from 24° to 100° F. requiring approximately 2 hours was recorded, and this represents a rate of increase approximating 0.6° per minute. Operating with a cam cut to a curve which approached the greatest usable curvature, the maximum useful rate of increase approximated 15° F. per hour or 0.25° per minute over the entire temperature range.

Figure 5 shows portions of thermograph records taken during a recent climate response test. *Aphytis* parasites were first subjected to the conditions pictured in figure 5 (1) for one generation and then to conditions shown in figure 5 (2) for a generation. These photos illustrate the excellent program reproducibility possible with this unit.

Humidity control within this cabinet has received only limited consideration. When a temperature curve from a field station is being duplicated, the relative humidity curve automatically obtained within the cabinet is not vastly different from the corresponding field curve. The only major difference occurs at the point when the temperature starts to rise. Instead of falling, as field relative humidity frequently does with a rising temperature, cabinet relative humidity climbs sharply to saturation and remains there for a brief period (because of defrosting of the refrigeration coils as the temperature starts up from a low) before it falls back and resumes its normal downward trend.

Several possibilities exist for modifying the humidity response to varying temperature within the cabinet but are yet to be explored. A saturated salt bath in a shallow basin should help in smoothing the humidity curve if the temperature swings are not too great. A method utilizing a special two-compartment chamber built into the recirculation duct might be useful. One compartment would contain either an array of atomizers or a steam jet for humidification of the recirculated air while the second would contain a replaceable charge of calcium chloride for dehumidification of cabinet air. A third possibility would be the method described by Howe (1956). With comparatively simple apparatus, he was apparently able to achieve satisfactory humidity control at a constant level through the limited range of temperatures with which he was concerned.

Light control in the cabinet has not been necessary with the organisms we have tested but could be provided continuously by means of a small-wattage incandescent or fluorescent lamp. By adding a time clock, the time during which the light is on could be controlled. This system could be used then to provide light for a time correspond-

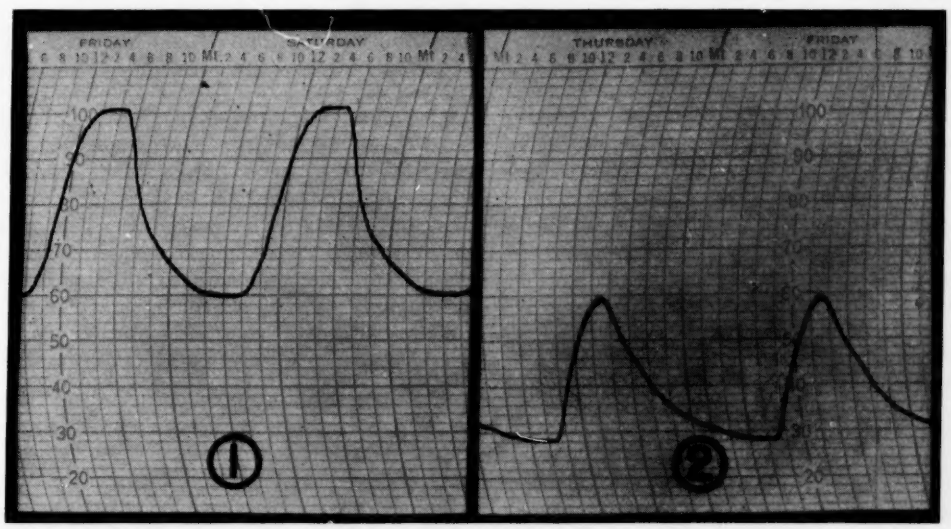

FIG. 5.—Two typical temperature response curves recorded in the variable temperature cabinet.

ing to the natural daylight period desired.

COST.—The cost of constructing a variable temperature cabinet may vary considerably, depending on the amounts paid for labor and for the refrigerator to be modified. Therefore, in the following estimates only the cost of materials required to perform this modification are considered.

Amount	*Item*	*Cost*
1	Linkage mechanism—custom made	$ 1.50
2	Electronic relays—American Instrument Co. ($42.50 ea.)	85.00
1	Bimetalic thermoregulator—"Quickset"—American Instrument Co.	20.00
1	Electric clock, 24-hour	12.00
1	Squirrel cage fan, 60 cu. ft./min.	8.50
1	Heating element	1.00
	Miscellaneous—including duct, electrical hook-up wire, power cord, etc.	10.00
	Total	$138.00

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Systemic Insecticidal Action in the Cortical Tissues of Elm Twigs[1]

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ABSTRACT

Two vinyl phosphates which contain carboxyamide groupings (Shell 3562 (2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate) and phosphamidon) were found to be effectively localized in the cortical twig tissues of elm trees following trunk implantation. Shell 3562 prevented niches from exceeding 3.0 mm. for 15 and 29 days after treatment, respectively, with tree dosages of 6 and 18 gm. The method of assay involved feeding-niche length and beetle mortality of *Scolytus multistriatus* (Marsh.) when exposed to twigs from treated trees.

The properties responsible for the above action of the systemics are apparently one or both of the following: (1) a high water solubility and (2) a phosphate or phosphorothiolate rather than a phosphorothionate or phosphorodithioate compound.

The smaller European elm bark beetle, *Scolytus multistriatus* (Marsh.), transmits the Dutch elm fungus, *Ceratocystis ulmi* (Buis.) Moreau, through feeding niches in elm twigs. In seeking to prevent such infection by treatment of elms with systemic insecticides, the chemical must be deposited in the bark in quantities sufficient to kill or repell the beetles before their niches reach approximately 3 mm. in length (Al-Azawi & Norris 1959). Initial research on the systemic approach to killing this beetle and reducing its extent of twig feeding in elm was by Al-Azawi & Casida (1958). Chipman R-6199 (*O,O*-diethyl *S*-(β-diethylamino)ethyl phosphorothiolate (hydrogen oxalate salt)) was the most promising systemic used by those workers. The first experimental prevention of fungus infection of elms by systemic control of beetle feeding in such trees was with R-6199 (Al-Azawi & Norris 1959). Since this work, the chemical and physical properties of R-6199 have been compared with those of the other previously tested systemics, such as phorate, demeton and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate), that failed to show promise in the work of Al-Azawi & Casida (1958). Two differences noted were (1) 50% water solubility and (2) presence of an amide grouping in R-6199 and not in the others (Norris 1959). In order to study further the importance of these properties in rendering a compound effective, two systemics possessing these properties, Shell 3562 (2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate) and phosphamidon, were studied in comparison with Di-Syston, demeton and phorate which lack both characteristics. In addition, dimethoate was investigated as a compound having an amide grouping and limited water solubility (7%). The results of the investigations are presented in this paper.

METHODS.—On August 11, 1958, 20 gm. of technical Shell 3562, Di-Syston, phorate or demeton were implanted in each of three 3-inch d.b.h. slippery elm trees, *Ulmus rubra* Mühl. This was accomplished by placing one-third of the total dosage in each of three 0.5-inch drilled holes located 6 inches above the soil surface on the main trunk. The holes were slanted downward at an approximately 45° angle to the main axis of the trunk and plugged with corks after implantation of the chemicals. Drilled, distilled-water-treated trees were used as controls.

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This research was supported in part by the Wisconsin Conservation Department and Shell Development Company.

These data were presented in part at the Annual Meeting of the Entomological Society of America, held at Detroit, Michigan, November 30–December 3, 1959. Accepted for publication May 16, 1960.

At 1, 3, 7 and 21 days after treatment, three random branch samples were taken from the crown of each tree. From these, five twig sections, approximately 3, 5, 7, 9 and 11 mm. in diameter and each containing five crotches, were taken and placed in a new 1-gallon cardboard ice cream carton with 10 newly emerged *S. multistriatus* beetles for a 24-hour bio-assay. At the conclusion, data on beetle mortality, number of feeding niches, number of niches to the cambium and length (longest dimension) of feeding niches in mm. were obtained.

On May 5, 1959, Shell 3562 (6 or 18 gm. per tree), phosphamidon and dimethoate (10 or 20 gm. per tree) were each implanted in three 3-inch d.b.h. forest inhabiting American elms, *Ulmus americana* L., in the same manner as described above (except the 20-gm. dosage of phosphamidon, which was a soil-surface application ringing the root crown). Three drilled, distilled-water-treated trees were used as controls. At 9, 15, 22, 29, 36, 60 and 65 days after treatment, three terminal branches were taken at random from each test tree. From these branches of each tree, five twig sections (as above) were chosen. These were then bioassayed as above except the duration of the assays was 48 rather than 24 hours.

All trees were observed for symptoms of phytotoxicity.

RESULTS AND DISCUSSION.—Shell 3562 and phosphamidon were more effective in causing beetle mortality and reducing feeding niche length than the other chemicals (tables 1 and 2). In bioassays of twigs from Shell 3562-treated trees (table 1), the beetle mortality was 75% or more through 21 days after treatment. The residual effectiveness of this treatment was determined only through 21 days after treatment. Mortality in assays from other 1958 treatments did not exceed 53.3%. In 1959 studies, both Shell 3562 and phosphamidon (as trunk implantation) caused highly singificant (99% level) beetle mortality in the assays at 9 days (table 2). The data for 3562 indicate a degree of beetle mortality exceeding 75% for at least 65 days at 18 gm. and 36 days at 6 gm. per tree. The period of such control at 6 gm. probably exceeded 36 days, but the next assay at 65 days showed the mortality was below 75%.

In terms of preventing Dutch elm fungus infection of elms through the feeding niches of *S. multistriatus* by treatment of the trees with systemic insecticides, the reduction in length of the niches is the most important consideration. Shell 3562 and phosphamidon gave the largest reductions in the niche length (tables 1 and 2). With the exception of one of three trees at 9 days after treatment with 18 gm., niches 3.0 mm. or longer did not occur in assayed twigs from trees treated with 6 or 18 gm. of Shell 3562 until 22 and 36 days after treatment, respectively. The poor control of feeding in the one tree at 9 days can be attributed to exceptionally slow translocation of the chemical in the tree, since at 15 days and thereafter the results in all three replicates were uniform. With our present knowledge, the number of niches shorter than approximately 3.0 mm. is not important in the disease infection situation. However, Shell 3562 did give as much as a 75% reduction in the number of niches reaching the twig cambium (table 2). This occurred with the 18 gm. dosage at 29 days after treatment. Dimethoate did not give a significant reduction in niche length (table 2).

The LD_{50}'s of Shell 3562 and phosphamidon to the beetles are not as low as those for some of the other insecticides tested, but the effectiveness of these two chemicals in reducing niche length was significantly better. The effectiveness of these compounds can be attributed to one or both of the following properties (1) high water solubility and (2) a phosphate or phosphorothiolate structure. The presence of amide groupings may be important in regard to water solubility of some effective compounds. Of the tested compounds, these two properties are both present only in Shell 3562, phosphamidon and Chipman R-6199. If water solubility is required the minimum adequate degree is not known, but must exceed 7.0% (the solubility of dimethoate).

The differences in the residual effectiveness of phosphamidon, Shell 3562 (tables 1 and 2)) and R-6199 (Al-Azawi & Norris 1959) in twig bark probably may be attributed mostly to difference in metabolic stability of the compounds in the elm plant; however, this stability has been studied thoroughly only with R-6199.

In terms of phytotoxicity, yellowing of leaves increased with the dosage of Shell 3562, but also varied with the season. At 20 gm. in August, phytotoxicity was complete on the leaves, but ranged from 0% to 30% per tree at 18 gm. in May. Six grams per tree caused only 0% to 5% in May. Since the tree protection period against *S. multistriatus* must extend about 30 days in Wisconsin, the necessary dosage per 3-inch d.b.h. tree should approximate 18 gm. of the mixture of Shell 3562 isomers. However, the alpha isomer is the highly insecticidal component of the mixture. Tests in the laboratory have indicated a considerable increase in beetle mortality and reduction in niche length per gram of alpha isomer as compared with the mixture. Such an increased effectiveness per gram of chemical could allow the total tree dosage to be reduced; perhaps to the point where phytotoxicity is insignificant. Tests to compare the alpha isomer and the mixture on a gram-for-gram basis in forest-inhabiting elms are being initiated. Phosphamidon and dimethoate also caused various degrees of phytotoxicity

Table 1.—Beetle mortality and reduction of number and length of beetle-feeding niches in twigs from elms treated with 20 grams of the active ingredient of various systemic insecticides, 1958.

Treatment	Days After Treatment	Beetle Mortality After 24 Hours (%)	No. Niches	No. Niches to Cambium	Length of Cambium Niches	
					Range (mm.)	Mean (mm.)
Di-Syston	1	33.3	33	28	1.0–11.0	4.5
Demeton	1	53.3	30	27	1.0– 9.0	4.3
Phorate	1	21.7	48	44	1.0–10.0	4.8
Shell 3562	1	93.7	14	1[a]	1.0	1.0
Control	—	21.7	38	30	1.0–10.0	4.7
Di-Syston	3	33.3	27	22	1.0– 9.0	4.9
Demeton	3	46.7	9	8	1.0– 7.0	4.6
Phorate	3	30.0	28	17	1.0– 9.0	5.0
Shell 3562	3	75.0	16	5[a]	1.0– 2.0	1.3
Control	—	28.3	38	33	1.0– 9.0	5.2
Di-Syston	7	31.7	37	33	1.0–10.0	5.1
Demeton	7	48.3	29	25	1.0– 9.0	4.7
Phorate	7	33.3	41	35	1.0–10.0	4.9
Shell 3562	7	91.7	22	8[a]	1.0– 2.0	1.4
Control	—	23.3	53	43	1.0–11.0	5.0
Shell 3562	21	86.7	19	6[a]	1.0– 1.5	1.3
Control	—	26.7	46	40	1.0–10.0	5.3

[a] On the basis of our present knowledge of the required niche length for fungus infection, these niches would not result in such infection.

Table 2.—Beetle mortality and reduction of number and length of beetle-feeding niches in twigs from elms treated with various systemic insecticides, 1959.

Treatment	Grams of Active Ingredient	Days After Treatment	Beetle Mortality 48 Hours (%)	No. Niches	Niches to Cambium		Mean Niche Length	
					Number	Range in Length (mm.)	Total (mm.)	Cambium (mm.)
Shell 3562	6	9	91.2	22	17	1.0– 2.0	1.1	1.2
	18	9	80.0	18	8	1.0– 8.0	1.8	3.3
Dimethoate	10	9	27.5	16	16	1.0– 7.0	3.2	3.2
	20	9	20.0	24	19	1.0–13.0	4.2	5.2
Phosphamidon	10	9	63.3	18	17	1.0– 4.0	2.5	2.6
	20	9	23.3	16	13	1.0–10.0	3.7	4.9
Control	—	—	17.2	18	14	1.0–10.0	3.7	4.6
Shell 3562	6	15	100.0	18	7	1.0	0.5	1.0
	18	15	96.8	11	5	1.0– 2.0	0.8	1.2
Dimethoate	10	15	20.0	32	28	1.0– 7.0	3.4	3.9
	20	15	46.6	20	19	1.0– 6.0	3.6	3.7
Phosphamidon	10	15	40.0	21	19	1.0– 4.0	2.1	2.3
Control	—	—	13.3	19	19	1.0– 7.0	3.8	3.8
Shell 3562	6	22	86.7	14	12	1.0– 4.0	1.8	2.0
	18	22	100.0	24	10	1.0– 2.5	0.8	1.3
Dimethoate	10	22	40.0	26	23	1.0– 8.0	3.7	4.1
	20	22	36.6	22	22	2.0– 7.0	4.5	4.5
Phosphamidon	10	22	40.0	27	22	1.0–12.0	3.1	3.7
	20	22	50.0	28	20	1.0– 7.0	2.8	3.6
Control	—	—	26.7	34	26	1.0– 7.0	3.7	4.6
Shell 3562	6	29	100.0	19	13	1.0– 6.0	1.7	2.5
	18	29	100.0	12	5	1.0– 2.0	0.8	1.2
Dimethoate	10	29	40.0	22	21	1.0– 7.0	3.8	4.0
	20	29	40.0	25	21	1.0– 8.0	3.4	3.9
Phosphamidon	10	29	36.6	23	17	1.0– 7.0	3.1	4.0
	20	29	50.0	25	14	1.0– 8.0	2.7	4.6
Control	—	—	29.9	28	20	1.0–12.0	4.5	6.2
Shell 3562	6	36	90.0	30	25	1.0– 8.0	3.2	3.8
	18	36	96.7	14	13	1.0– 4.0	2.2	2.4
Control	—	—	26.6	26	25	1.0–10.0	5.2	5.3
Shell 3562	18	60	100.0	14	10	1.0– 5.0	2.1	2.9
Control	—	60	23.3	27	19	1.0– 9.0	3.4	4.7
Shell 3562	6	65	63.3	33	17	1.0–10.0	2.3	4.4
	18	65	76.6	30	13	1.0– 7.0	1.5	3.2
Control	—	65	26.7	28	23	1.0– 9.0	4.2	5.0

in elm foliage. These aspects of phosphamidon will be studied further in subsequent experiments.

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Animal Systemic Activity, Metabolism, and Stability of Co-Ral (Bayer 21/199)[1]

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Abstract

Co-Ral® (Bayer 21/199) (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate) was more stable in several species of insects than in mammals. Metabolites isolated from insects and mammals consisted of the oxygen analog, *O*-ethyl and *O,O*-diethyl phosphoric acid, *O,O*-diethyl phosphorothioic acid, and possibly "desethyl" Co-Ral. The cumarinyl ring structure was not opened *in vitro* as was demonstrated by alkaline degradation. The same metabolites were recovered from insects and rats but different quantities of each metabolite were formed. The ability of rats to degrade Co-Ral to water-soluble products more rapidly and completely than insects is probably a significant factor in determinining the selective toxicity of Co-Ral.

The mode of action and biochemistry of animal systemic insecticides must be thoroughly investigated before the materials can be used with maximum safety and efficiency. Bayer 21/199 (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate), referred to in this paper by its registered trade name Co-Ral®, is an effective animal systemic insecticide when applied dermally (Smith & Richards 1954, Roth & Eddy 1955) but ineffective when administered orally (McGregor *et al.* 1954, Adkins *et al.* 1955). Following oral administration, Co-Ral was rapidly degraded to water-soluble metabolites and excreted in the urine and feces of rats (Lindquist *et al.* 1958) and steers (Kaplanis *et al.* 1959). Co-Ral was absorbed slowly through the skin of rats, a cow, a goat (Krueger *et al.* 1959), steers (Kaplanis *et al.* 1959), and bulls (Robbins *et al.* 1959a) and the radiolabeled materials absorbed in the blood were water-soluble metabolites (Kaplanis *et al.* 1959, Robbins *et al.* 1959b). Thus, the effectiveness of dermal applications of Co-Ral against the cattle grub, *Hypoderma lineatum* (De Vill.), in the gullet of cattle (Brundrett *et al.* 1957) cannot be explained on the basis of Co-Ral transport by the blood. The selective toxic action of Co-Ral has been explained by differences in activation and deactivation processes in insect and mammalian tissues (O'Brien & Wolfe 1959).

The toxicity of Co-Ral and related derivatives to insects and mammals and the animal systemic effectiveness of these materials were investigated. The absorption, excretion, and metabolism of radiolabeled Co-Ral in insects and mammals are also reported in this paper.

Materials and Methods.—*Preparation of P-32 Co-Ral.*—Co-Ral was prepared from radiophosphorus thiophosphoryl chloride (10 mc, Volk Radiochemical Company) as the starting material. *O,O*-diethyl thiophosphoryl chloride was prepared by the general method of Fletcher *et al.* (1952) and the final reaction with chloroferron by the method of MacDougall (1958). The final product was chromatographed twice on a Celite partition column and then recrystallized from a 1:1 chloroform and *n*-hexane mixture. The melting point, chromatographic behavior, and infrared spectrum of the synthesized radiolabeled material were identical to those of an analytical standard sample of nonradioactive Co-Ral. The overall yield was 43% of theoretical, and the specific activity was 500 c.p.m. per microgram (end window G-M tube).

Chloroferron (3-chloro-4-methylumbelliferone), Potasan® (*O,O*-diethyl *O*-(4-methylumbelliferone) phosphorothioate), and the oxygen analog of Co-Ral were provided for this study by the Chemagro Corporation.

Animal Systemic Activity.—The bed bug (*Cimex lectularius* L.) and the Gulf Coast tick (*Amblyomma maculatum* Koch) were utilized to evaluate the animal systemic activity of Co-Ral and Potasan. Rabbits were treated orally, dermally, subcutaneously, or intramuscularly with Co-Ral and orally or dermally with Potasan. The numbers of animals treated, dosages in mg./kg., numbers of insects tested, and mortality data are shown in table 1. Methods used in rearing and handling test arthropods and handling and treating host animals were the same as those described by Adkins *et al.* (1955).

Table 1.—Animal systemic activity of Co-Ral and Potasan after administration to rabbits.

Compound	Route of Administration[a]	Dose (mg./kg.)	Per Cent Mortality After Feeding[b]	
			Bed Bug[a]	Gulf Coast Tick[a]
Co-Ral	Oral (2)	50	0 (46)	0 (32)
Potasan	Oral (2)	15	0 (53)	0 (39)
Co-Ral	Dermal (12)	200	100 (241)	0 (196)
Potasan	Dermal (2)	200	100 (38)	0 (43)
Co-Ral	Subcutaneous (4)	40	20 (79)	0 (101)
Co-Ral	Intramuscular (3)	50	0 (61)	0 (69)

[a] Numbers in parentheses indicate number of rabbits treated or number of arthropods tested.

[b] Bed bug mortality determined from number that died 24 hours after taking a blood meal; tick mortality determined from number that died on host.

Toxicity to Insects and Rats.—The relative toxicity of Co-Ral, oxygen analog of Co-Ral, chloroferron, and Potasan were compared from LD_{50} determinations made with adult 3-day-old female house flies (*Musca domestica* L.) adult bed bugs, fourth-instar larvae of the yellow-fever mosquito (*Aedes aegypti* (L.)) and mixed sexes of white rats. The compounds, dissolved in acetone, were applied in 1-μl. quantities to the abdomen of house flies and bed bugs; mortality counts were made 24 hours after treatment. From 6 to 10 replicates of 10 insects each were treated at each dosage level, and the LD_{50} values were calculated (Litchfield & Wilcoxon 1949). The toxicities of Co-Ral and related derivatives to yellow-fever mosquito larvae were determined from 24-hour mortality counts after the introduction of 10 larvae (3 to 6 replicates) into

[1] This investigation was supported in part by a research grant PHS E-549 from the Division of Research Grants of the National Institutes of Health, Public Health Service.

Taken from a thesis presented by the senior author in June, 1959, to the graduate faculty, Auburn University, in partial fulfillment of requirements for the M.S. degree in entomology. Accepted for publication May 16, 1960.

10 ml. of water containing the desired amount of insecticide (formulated as a wettable powder or water emulsion).

White rat LD_{50} values were determined after oral or subcutaneous administration of Co-Ral and related derivatives in a 1:1 mixture of corn oil and acetone. The appropriate amounts of insecticides corresponding to the weight of each rat (about 200 gm. each) were administered orally through an intramedic polyethylene stomach tube or subcutaneously with a 1-cc. syringe and No. 21 needle. After the approximate LD_{50} value was determined, about 10 additional rats were treated at or near this dosage level, and the final LD_{50} values were estimated by gross inspection of the data.

Insect Metabolism.—The metabolism of radiolabeled Co-Ral was studied in the house fly, German cockroach (*Blattiella germanica* (L.)) and stable fly (*Stomoxys calcitrans* (L.)). Two groups of 200 insects of each species were treated topically at 50 mg./kg. At 1 or 4 hours after treatment, each group of insects was surface-washed in acetone to remove unabsorbed insecticide. The whole insects were macerated in 20 ml. of water with a Waring Blendor equipped with a microhead attachment. The macerate was then extracted six times with equal volumes of chloroform using centrifugation to separate the two phases after each extraction. The chloroform extracts were combined and dried over anhydrous sodium sulfate, and the percentage of radioactivity partitioning from water into chloroform was calculated. The chloroform was evaporated, and the radioactive materials in the oily residue was co-chromatographed on Celite with nonradioactive Co-Ral and the oxygen analog to establish the presence and identity of metabolites.

The solvent system used in Celite partition chromatography consisted of acetonitrile saturated with a 1:1 mixture of *n*-heptane and cyclohexane as the stationary phase and a 1:1 mixture of *n*-heptane and cyclohexane saturated with acetonitrile with 3% chloroform (v/v) added to the final volume as the mobile or developing solvent. Phosphorus-containing materials in the column eluate were located by analyzing for phosphorus colorimetrically (Allen 1940) and radiometrically. Characterization by co-chromatography was determined by the differences in the sensitivity of the two methods for determining phosphorus. Co-Ral was eluted from the column with 260 to 380 ml. of solvent and the oxygen analog with 400 to 480 ml.

Rat Metabolism.—White rats (4 replicates of 2 rats each) were treated orally at 10 mg./kg. and dermally at 100 mg./kg. with radiolabeled Co-Ral. For either route of administration, the Co-Ral was mixed with corn oil and acetone (1:1). The radiolabeled Co-Ral was administered orally through a stomach tube and dermally through open-bottom screw cap vials which were glued to a shaved spot on the back of the rat. Excreta from each replicate of rats was collected (Comar 1955) at 24-hour intervals for 7 days following treatment.

An aliquot of the urine and an aqueous macerate of the feces (usually 0.1 ml.) were assayed for total radioactive materials to calculate the percentage of the administered dose that was excreted. The feces macerate and the urine were extracted six times with an equal volume of chloroform to remove Co-Ral, the oxygen analog, or other chloroform-soluble materials. These chloroform-soluble radioactive materials were co-chromatographed on Celite in the same manner as described for insects.

The water-soluble hydrolytic products (after chloroform extraction) from Co-Ral degradation were subjected to anion exchange co-chromatography with known *O*-ethyl phosphoric, *O,O*-diethyl phosphoric, and *O,O*-diethyl phosphorothioic acids. The solvent system for developing the chromatogram (Plapp & Casida 1958) consisted of three elution gradients: pH 3–2, pH 2–1, and pH 1–0. The pH 1–0 was mixed with methanol at a ratio of 1:3.

Two replicates (4) of the rats treated orally at 10 mg./kg. and dermally at 100 mg./kg. were sacrificed 3 days after treatment, and the remaining replicates of each were sacrificed at 7 days after treatment. The total p.p.m. of radioactive materials and the total p.p.m. of chloroform-soluble radioactivity occurring in the blood, brain, fat, heart, kidney, liver, muscle, and skin of each rat was determined. The chloroform-soluble radioactive materials representing the toxic residues were calculated from the proportionate amount of radioactivity which partitioned from a macerate of each tissue into chloroform. When the tissues were analyzed, the limit of sensitivity of the radiometric method was about 0.01 p.p.m.

Two rabbits were treated orally at 10 mg./kg. and two were treated dermally at 100 mg./kg. with P-32 Co-Ral. At 2, 4, 8, 14, 24, and 36 hours after treatment, blood samples were taken from the ear of each rabbit. The flow of blood from the clipped ear of the rabbit was facilitated by massaging with a 1:1 mixture of isopropyl alcohol and xylene; a small pinch clamp was used to stop the flow. Blood (0.5 ml.) samples were made to 3 ml. volume with distilled water and 3 ml. chloroform was added. Radioassays of each phase were used to calculate the percentage of hydrolytic and nonhydrolytic products. One rabbit treated orally and one treated dermally were sacrificed after 3 days, and the remaining two were sacrificed at 7 days after treatment. Several tissues were analyzed for total radioactivity and chloroform-soluble radioactivity in the same manner as described for rat tissues.

In Vitro Studies.—The *in vitro* metabolism of radiolabeled Co-Ral was studied, using rat liver slices (DuBois *et al.* 1950). Four flasks containing 0.2 mgm. of Co-Ral-32 were incubated under an oxygen atmosphere at 37° C. for 1 hour on a metabolic reciprocating shaker and four flasks were incubated for 4 hours. After each incubation period, the buffered aqueous medium (pH 7.2) was extracted six times with equal volumes of chloroform that were combined, dried, and concentrated, and the residue was chromatographed on Celite with known carrier Co-Ral and oxygen analog. The percentage of hydrolytic products was calculated from the radioactive materials remaining in the water after chloroform extraction plus the radioactivity remaining in the liver residue.

The ability of human serum esterases to hydrolyze Co-Ral was also investigated (Arthur & Casida 1957). Human blood serum was buffered at pH 7.0 and 50 p.p.m. P-32 Co-Ral was added. After 0, 1, 2, 3, 4, and 8 hours of incubation at 37° C., an aliquot was partitioned with an equal volume of chloroform to derive the percentage hydrolysis. Each determination was run in duplicate for each incubation time interval.

Chemical Studies.—In alkaline degradation studies 1

gm. of recrystallized Co-Ral was dissolved in 25 ml. of ethyl alcohol which was added to 100 ml. of 0.1 N potassium hydroxide and heated for 1 hour at 92° to 95° C. Unreacted Co-Ral was removed by extracting the mixture three times with equal volumes of chloroform. The nature of the water-soluble hydrolytic products was studied by adjusting the alkaline solution to pH 1 and partitioning again with chloroform. Phosphorus-containing materials that partitioned into chloroform after lowering the pH were studied further by chromatographic and infrared analyses.

Bromine water oxidation of Co-Ral to form the oxygen analog was accomplished by the methods described by Fallscheer & Cook (1956). The chloroform extracts of the mixture were subjected to chromatographic and infrared analyses.

The volatility of Co-Ral was determined by loss of total phosphorus (Allen 1940) at room temperature from 200 μg. placed in 1-ml. beakers. Duplicate phosphorus determinations were made at 24-hour intervals for 12 days. The thermal stability of Co-Ral was determined by heating 500 mg. in sealed ampules at 120° to 170° C. for 1 to 3 hours and noting the changes in infrared spectra and solubility changes in carbon tetrachloride and chloroform.

RESULTS AND DISCUSSION.—*Animal Systemic Activity of Co-Ral and Potasan.*—Co-Ral and Potasan administered orally to rabbits at 50 and 15 mg./kg., respectively, were not systemically effective against the bed bug or the Gulf Coast tick (table 1). These data on Co-Ral administered orally to mammals substantiate results reported by McGregor *et al.* (1959) and Adkins *et al.* (1955); however, Drummond (1958) reported systemic activity against the stable fly and screw-worm larvae feeding on guinea pigs treated orally with Co-Ral.

When applied dermally at about 200 mg./kg., Co-Ral and Potasan were systemically effective against the bed bug but not against the tick (table 1). Bed bugs that did not feed on the host molted normally, indicating that mortalities from those that completed a blood meal resulted from systemic activity and not contact toxicity. The highest mortality of bed bugs occurred at 6 to 10 hours following dermal treatment of two rabbits with Co-Ral, while with two other rabbits the highest mortality was observed at 24 hours after treatment. From the radiotracer studies, the highest concentration of radiolabeled phosphorus compounds was present in the blood of rabbits at 24 to 30 hours after treatment (fig. 1).

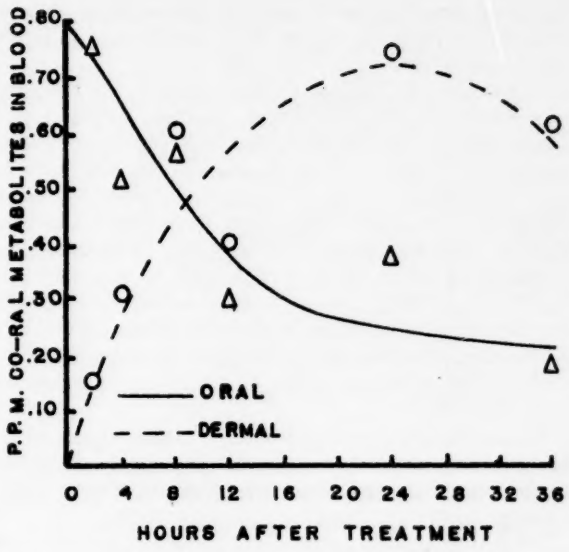

FIG. 1.—Concentration of P-32 Co-Ral and metabolites in the blood of rabbits following treatment orally at 10 mg./kg. and dermally at 100 mg./kg.

Three rabbits treated intramuscularly at 50 mg./kg. exhibited no systemic activity against the bed bug or tick at 1, 2, 4, 8, or 24 hours after administration (table 1); subcutaneous treatments were also ineffective. Ticks feeding on rabbits treated with Co-Ral or Potasan by either route of administration engorged, dropped from the host, and molted normally.

Toxicity of Co-Ral and Related Derivatives.—Co-Ral and its oxygen analog were about equally toxic to the house fly when applied topically, while Potasan was about one-third as toxic (table 2). The oxygen analog of Co-Ral was less toxic to the bed bug than either Co-Ral or Potasan. Co-Ral was 16 times more toxic to yellow-fever mosquito larvae than the oxygen analog and 26 times more toxic than Potasan. This apparent increase in toxicity of a phosphorothioate over its corresponding oxygen analog is somewhat unusual (Metcalf 1955); however, the isotoxic nature of Co-Ral and the oxygen analog to house flies has been noted (Monroe & Robbins 1959).

Potasan, lacking a chlorine atom at the number 3 position of the cumarinyl ring structure as compared with Co-Ral, was less toxic than Co-Ral to house flies, bed

Table 2.—Toxicity and anticholinesterase activity of Co-Ral and related derivatives.

COMPOUND	LD_{50} MG./KG.			LC_{50}	
		Topical			
	Oral Rats	House Fly	Bed Bug	P.p.m. *Aedes* Larvae	MOLAR CONCENTRATION 50% ChE Inh.[a]
Co-Ral	20	$10.1^{\pm 1.5}$	$1.1^{\pm 0.30}$	$0.03^{\pm 0.02}$	1.36×10^{-4}
O-analog	11	$10.2^{\pm 1.8}$	$2.8^{\pm 0.72}$	$0.48^{\pm 0.22}$	2.28×10^{-7}
Chloroferron	>1,000	>2,000	>2,000	>500	$>2.33\times10^{-4}$
Potasan	20	$29^{\pm 2.2}$	$1.5^{\pm 0.31}$	$0.80^{\pm 0.20}$	1.54×10^{-4}
Co-Ral	Subcutaneous 285				

[a] Molar concentration of inhibitor causing 50% inhibition of cholinesterase of 30% whole human blood, *in vitro* (Hensel *et al.* 1954).

bugs, and mosquito larvae (table 2). Chloroferron was not toxic to the three species of insects at the dosage levels shown in table 2.

The acute oral LD_{50} of Co-Ral and Potasan to white rats was 20 mg./kg.; the oxygen analog of Co-Ral was about twice as toxic (table 2). This oral toxicity of Co-Ral to rats was determined from treatment of 64 rats and was quite comparable to the toxicity of Co-Ral to mice (Robbins *et al.* 1959b, O'Brien & Wolfe 1959), and the highest sublethal dose of 25 mg./kg. to guinea pigs (Drummond 1958). This oral toxicity to rats was five times greater than the highest sublethal dose of 100 mg./kg. to rabbits (Adkins *et al.* 1955). When Co-Ral was administered orally at 50 mg./kg. to rabbits in connection with animal systemic studies, no symptoms of poisoning were observed.

Co-Ral was considerably less toxic to rats when administered subcutaneously than when given orally (table 2). Co-Ral was apparently nontoxic to rabbits at 40 mg./kg. subcutaneously or 50 mg./kg. intramuscularly.

No toxic symptoms were noted when rats were treated dermally with Co-Ral at 40, 100, or 500 mg./kg. However, the absorbing area was no more than 10% of the total skin surface. When technical Co-Ral was applied to the shaved backs of rabbits at 500 mg./kg., one out of three rabbits died. Two out of five rabbits sprayed with 0.75% Co-Ral from a wettable powder died within 3 days following treatment.

Metabolism by Insects.—From 15% to 30% of the Co-Ral applied topically to German cockroaches and house flies was absorbed within 4 hours after treatment. In house flies about 25% of the absorbed Co-Ral was degraded to water-soluble hydrolytic products, while only 10% was degraded by cockroaches during the first 4 hours after treatment. Most of the radioactive materials recovered from an aqueous macerate of house flies, German cockroaches, or stable flies with chloroform was unchanged Co-Ral (table 3). More oxygen analog was present in German cockroaches 4 hours after treatment than at 1 hour. Another radioactive material(s) that was extractable from water with chloroform was eluted from the Celite column with methyl alcohol. The nature of this material(s) was not determined. The *in vivo* activation of Co-Ral supports *in vitro* studies with insect tissues (O'Brien & Wolfe 1959), but degradation of Co-Ral was more prevalent *in vivo* than *in vitro*.

Table 3.—Fractionation of radioactive Co-Ral and metabolites recovered from several biological systems.

Biological System	Type of Treatment	Time After Treatment	Per Cent Present as[a]		
			Co-Ral	Oxygen Analog	Methanol Fraction
House fly	Topical	1 hour	99.1	0.6	0.3
German roach	do	1 hour	98.7	0.1	1.3
	do	4 hours	96.5	3.4	0.1
Stable fly	do	1 hour	91.0	0.1	8.9
Rat urine	Oral	1 day	17.7	39.5	42.8
	do	2 days	30.8	68.0	1.2
	do	3 days	25.6	74.4	0.0
	Dermal	1 day	21.9	22.9	55.2
	do	2 days	24.3	65.6	10.2
	do	3 days	30.5	59.7	9.9
Rat feces	Oral	1 day	57.5	38.0	4.5
	do	2 days	42.3	57.8	0.0
	Dermal	1 day	71.9	28.1	0.0
	do	2 days	72.5	27.5	0.0
	do	3 days	32.6	67.4	0.0
Human plasma	In vitro	4 hours	100.0	0.0	0.0
Rat liver	In vitro	1 hour	94.2	4.2	5.6
	In vitro	4 hours	62.6	33.2	4.3

[a] Identification of residual Co-Ral and the oxygen analog based on co-chromatography on Celite from single determinations except the excreta from rats treated orally was from duplicate chromatograms.

Mammalian Excretion, Metabolism, and Residues.—White rats treated orally or dermally with P-32 Co-Ral excreted more radioactive materials in the urine than in the feces (fig. 2). Of the 10 mg./kg. dose of Co-Ral administered orally, 54.7% was eliminated in the urine and 14.5% in the feces during the first 7 days following treatment. Only 3.74% was eliminated in the urine and 1.85% in the feces of rats treated dermally at 100 mg./kg. The Co-Ral applied dermally was absorbed by less than 10% of the total body surface of rats, and this may account for the small percentage of the administered material being absorbed through the skin and eliminated in the excreta. However, dermal applications usually result in small amounts of radioactivity in the excreta of mammals (Krueger *et al.* 1959, Robbins *et al.* 1959, Kaplanis *et al.* 1959).

Most of the radioactive materials eliminated in the urine and feces of orally or dermally treated rats were in the form of hydrolytic products as determined by exhaustive partitioning between chloroform and water (table 4). By either route of administration, the amount of chloroform-soluble radioactive materials was never greater than 6.9% in the urine or 21.2% in the feces. Co-chromatography on Celite of the chloroform-solubles recovered from urine and feces yielded residual Co-Ral, the oxygen analog, and a methanol fraction (table 3, fig. 3). There was more residual Co-Ral in the feces than in the urine of rats treated by either route of administration. The percentage of oxygen analog was greater in the urine when Co-Ral was administered orally than when administered dermally. Although the percentage of oxygen analog recovered from the urine appeared quite high as compared with residual Co-Ral (table 3), the total chloroform-soluble radioactive products represented less than 7% and usually only 2% or 3% (table 4) of the total output of radiolabeled materials during a given 24-hour period. Thus, the oxygen analog accounted for only 1.5% to 4% of the total radiolabeled materials excreted.

Another radioactive material that was extractable from water with chloroform was not eluted from Celite with the solvent system described for Co-Ral and the oxygen analog (see methods section on insect metabolism) or by increasing the percentage of chloroform in the developing solvent up to 50% (v/v). This material was eluted from Celite with methyl alcohol (table 3). The methanol fraction was more prevalent in the urine on the first day following treatment of rats either orally or dermally than on the second or third day after treatment. The feces contained less of the methanol fraction than the urine. By combining several methanol fractions from the chromatograms, a sufficient quantity of residue was obtained for infrared analysis. A broad OH band was present in the spectrogram at 3 μ (Bellamy 1956), which may have resulted from hydrolysis of the cumarinyl ring structure or from hydrolysis of an *O*-ethyl ester linkage to form "desethyl" Co-Ral (Krueger *et al.* 1959). The spectrogram of the methanol fraction was not like spectro-

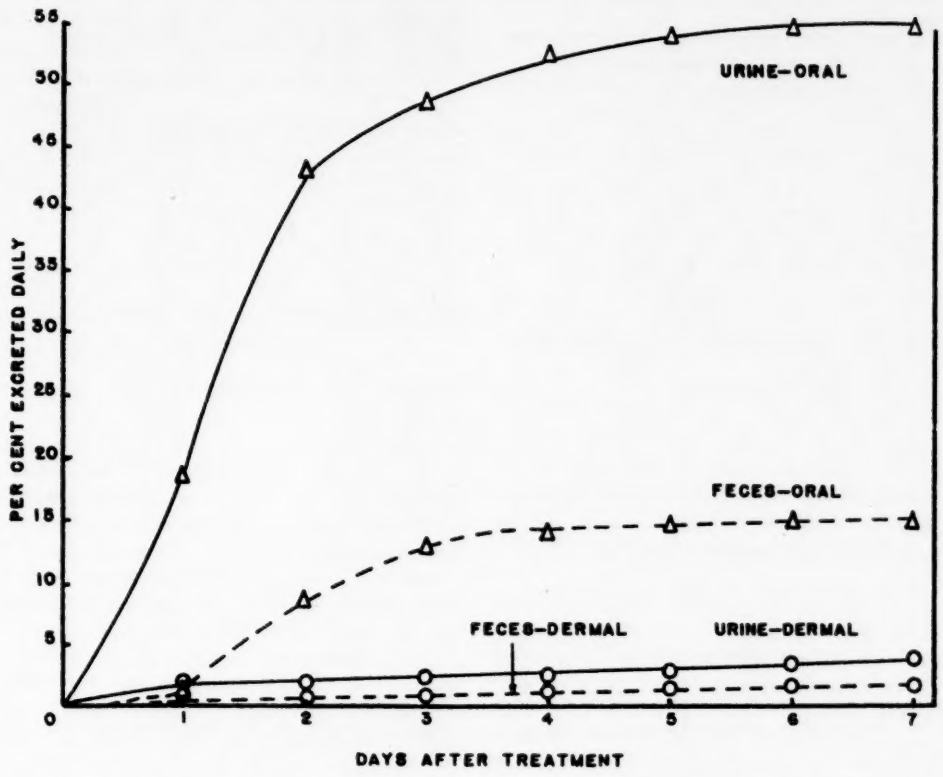

FIG. 2.—Excretion rates of P-32 Co-Ral and metabolites in the urine and feces of rats treated orally at 10 mg./kg. and dermally at 100 mg./kg.

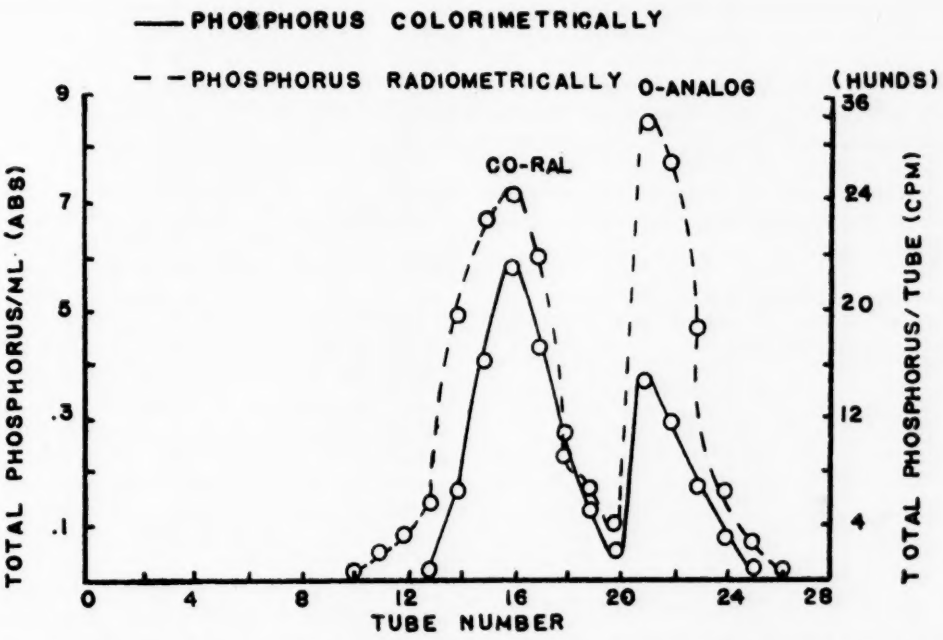

FIG. 3.—Co-chromatography on Celite of Co-Ral and the oxygen analog with the chloroform-soluble radiolabeled metabolites recovered from rat feces 1 day after dermal application of P-32 Co-Ral to rats at 100 mg./kg.

Table 4.—Hydrolysis of radioactive Co-Ral from white rats treated orally at 10 mg./kg.[a]

Days After Treatment	Per Cent Hydrolysis[b]			
	Oral		Dermal	
	Urine	Feces	Urine	Feces
1	92.9	78.8	97.1	93.6
2	96.8	94.8	93.1	97.3
3	97.3	98.0	98.8	99.4
4	100	100	98.2	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100

[a] Average of 4 replicates of 2 rats each.
[b] Based on chloroform/water partition coefficients.

grams of known diethyl phosphoric or phosphorothioic acid. The material was different from one produced by alkaline degradation of Co-Ral, which could not be extracted from water with chloroform until held at pH 1 for 30 minutes.

The hydrolytic products of Co-Ral degradation by rats as determined by co-chromatography on an anion resin were *O*-ethyl and *O,O*-diethyl phosphoric and *O,O*-diethyl phosphorothioic acids (table 5). For rats treated dermally, the ratio of *O*-ethyl and *O,O*-diethyl phosphoric acids to *O,O*-diethyl phosphorothioic acid occurring in the urine was about equal. Diethyl phosphorothioic acid was the predominant metabolite in the urine of rats treated orally. The presence of *O*-ethyl phosphoric acid may have been due to hydrolysis of the P-*O*-Ar bond of "desethyl" Co-Ral.

The p.p.m. of Co-Ral and metabolites in several tissues of rats and rabbits are shown in table 6. The kidney and liver from rats treated orally and the kidney, liver, and skin from rats treated dermally contained higher concentrations of total radioactivity than other tissues. Most of the radioactive components in the tissues were hydrolytic products that could not be extracted from a macerate of the tissues with chloroform. The kidney and skin were the only tissues containing measurable chloroform-soluble

Table 5.—Hydrolysis products of radioactive Co-Ral from rat urine and feces following oral and dermal administration at 10 and 100 mg./kg., respectively.

Excrement	Days After Treatment	Per Cent Present as[a]	
		$C_2H_5OP(O)(OH)_2$ + $(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$
Urine (oral)	1	15.8	84.2
	2	16.0	84.0
	3	8.2	91.8
	4	10.7	89.3
Feces (oral)	2+3	76.3	23.7
Urine (dermal)	1	46.6	53.4
	2	60.7	39.3
	3	48.1	51.9

[a] Identifications based on anion exchange chromatography with known carrier compounds.

Table 6.—Parts per million of radioactive Co-Ral and metabolites in tissues after oral (10 mg./kg.) and dermal (100 mg./kg.) application to rats and rabbits.

Tissue	P.p.m. Based on Total Radioactivity at Indicated Days							
	Rats (Oral)		Rats (Dermal)		Rabbits (Oral)		Rabbits (Dermal)	
	3	7	3	7	3	7	3	7
Blood	0.42	0.09	1.42	1.41	0.02	0.02	0.84	0.51
Brain	.14	.17	.28	.25	.06	.07	.18	.15
Fat	.20	.05	.89	1.10	.0	.0	.11	.27
Heart	.46	.25	.44	.58	.07	.08	.20	.39
Kidney	1.53	.52	1.85	1.62	.41	.61	1.26	1.92
Liver	3.25	.86	2.80	4.36	1.77	2.26	1.15	1.89
Muscle	.32	.13	.56	.37	.0	.02	.04	.17
Skin	.27	.53	2.65	.99	.05	.14	—	—

residues at 3 days following dermal administration of Co-Ral.

Residues in tissues from rabbits treated orally or dermally were quite comparable to those in rat tissues (table 6). The highest concentration of radioactive materials in the blood of rabbits occurred 2 hours after oral treatment and 24 hours after dermal treatment (fig. 1). None of the radioactive materials could be extracted from the blood with chloroform, indicating that the absorbed Co-Ral was rapidly degraded to hydrolytic products.

Comparison of Co-Ral Metabolism in Insects and Mammals.—The Co-Ral molecule has four sites subject to enzymatic degradation: (I) The formation of the oxygen analog by oxidation at the P=S followed by hydrolysis, (II) the formation of "desethyl" Co-Ral by hydrolysis at an *O*-ethyl ester linkage, (III) the degradation to diethyl phosphorothioic acid by hydrolysis at the P-*O*-Ar bond, and (IV) the hydrolysis at the number 2 position of the cumarinyl ring structure resulting in a carboxyl group to form "open ring" Co-Ral. Co-Ral was degraded as described in I and III, and possibly II (tables 3 and 5). No evidence was obtained that Co-Ral was degraded to "open ring" Co-Ral in insects or rats as was demonstrated in alkali (see section on chemical studies). Although the same metabolites were recovered from insects and rats, there were differences in the quantities of each metabolite formed. Only 3% to 6% of the radiolabeled Co-Ral administered orally or dermally to rats was recovered in the excreta as unchanged Co-Ral, the oxygen analog, or other chloroform-soluble metabolites (tables 3 and 4). In contrast, 90% of the dose absorbed by cockroaches and 75% of the dose absorbed by house flies was recovered as Co-Ral or chloroform-soluble materials. The other 94% to 97% of the radioactive materials from rat excreta, 10% from cockroaches, or 25% from house flies was recovered as water-soluble products as determined by partitioning between chloroform and water and by column chromatography. The ability of rats to degrade Co-Ral to water-soluble products more rapidly and completely than insects was probably a significant factor in determining the selective toxicity of Co-Ral.

In Vitro Studies.—Activation of Co-Ral to the more potent anticholinesterase agent, the oxygen analog (table 2), was demonstrated *in vitro* using rat liver slices (table 3). Both activating and degrading systems (O'Brien & Wolfe 1959) were present in rat liver.

Co-Ral was quite stable in the presence of human serum esterases; there was less than 10% degradation after 8

hours incubation. All of the chloroform-solubles recovered from the serum chromatographed as Co-Ral. The stability of Co-Ral in human serum suggests that the rapid degradation of Co-Ral in mammals was probably by the liver rather than the blood.

Chemical Studies.—Opening of the lactone ring of Co-Ral by alkali (Krueger *et al.* 1959, Kane *et al.* 1960) was substantiated by chromatographic and infrared analysis. However, there was no evidence that this alkaline degradation product was formed biologically.

Bromine water was effective in oxidizing 55% of a 1-gm. sample of Co-Ral to the oxygen analog while 43.5% was recovered as unchanged Co-Ral as determined by chromatographic and infrared analysis.

Co-Ral was nonvolatile when exposed at room temperature for 12 days. Heating Co-Ral in a constant temperature oven for 2 hours at 150° C. produced no changes in infrared spectra or noticeable changes in solubility of Co-Ral in carbon tetrachloride. An extra absorption band at 8.23 μ appeared in the spectra of Co-Ral heated for 2 hours at 170° C. while continued heating at this temperature for another hour produced a charred residue that was not soluble in carbon tetrachloride and only slowly soluble in chloroform. Co-Ral was more stable to heat than several other organophosphates (Metcalf 1955).

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Resistance of Pines to the Pine Reproduction Weevil, *Cylindrocopturus eatoni*[1]

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ABSTRACT

The pine reproduction weevil causes serious losses in young plantations of *Pinus jeffreyi* Grev. & Balf. and *P. ponderosa* Laws. Crossing *P. jeffreyi* with *P. coulteri* D. Don. produces resistant progenies. A three-way hybrid of these two and *P. ponderosa* is also resistant. The mode of action of resistance is not known though nonpreference, antibiosis, and tolerance all are indicated. Within-species variation in resistance appears to be heritable. Both within- and between-species resistance may be imparted by either parent, though indications are that it may be more attributable to the female parent. Progenies from self-pollination of recognized hosts are less resistant than progenies from non-selfing. Many pine species and hybrids have been classified as resistant or non-resistant from data of forced-attacks and natural infestations. A large-scale pollination project is underway to produce weevil-resistant pines for tree planting in California.

In many areas in California the pine reproduction weevil, *Cylindrocopturus eatoni* Buch., has been a major factor in the loss of planted seedlings of ponderosa pine, *Pinus ponderosa* Laws., and Jeffrey pine, *P. jeffreyi* Grev. & Balf. One of the approaches to control of the weevil has been the use of seedlings derived from hybridizing these two susceptible parents with a resistant parent, usually Coulter pine, *P. coulteri* D. Don.

This article has two purposes: (1) To present a brief review of the early research on resistance of pines to weevils, much of which has not been published; and (2) to record the significant results of recent experiments on this subject.

Research on the resistance of pines to the pine reproduction weevil was started by J. M. Miller in 1946, in cooperation with forest geneticists at the Institute of Forest Genetics, Placerville, California. The aim has been to try to incorporate resistance to the weevil into pine hybrids. The reproduction weevil was chosen for the work because it met two important requirements. It was, and still is, a serious pest of native pines in California. Secondly, it is a pest of young trees and therefore the resistance of the progeny of inter- and intra-specific hybridization could be determined within just a few years. The time element is a major obstacle in resistance work with trees; the life cycle, seed to seed, of many trees is 20 years or more.

The details of the weevil's life history were determined by Eaton (1942) and more recently have been summarized by Hall (1957). The weevil has one generation per year. It overwinters as a larva in the wood of dying or recently killed trees. The adult, a small weevil 2 to 3 mm. long, emerges in the spring and first feeds lightly on the needles of living pines, making punctures in the needles which show up as small target spots or bull's eyes. The weevil soon shifts to the stem and twigs where it makes numerous feeding punctures in the cortex. Small globules of resin form at some of these feeding punctures. A necrotic area, which is far more extensive than that which may be expected from the mechanical effects of feeding alone, develops around each puncture. This necrosis is largely owing to the fungus *Hormiscium gelatinosum* Hedgc., a common lumber stain associated with the weevil.

Eggs are laid in niches in the cortex. These hatch and the larvae first feed in the necrotic area of the cortical tissue. Later they break through the necrotic zone and move inward to the cambial layer. Here the feeding becomes extensive, girdling the tree so that it usually dies in the fall. Apparently, the success of the insect's development is intimately associated with the rapidity of death of the plant and with the fungus introduced by the weevil. Toward fall the larva bores into the woody tissue to overwinter. Attacks are confined to the stem and twigs below the current year's growth. Successful attacks are generally made on trees less than 6 feet in height; infrequently a taller tree may be attacked and killed.

METHODS.—Two procedures have been used in determining the resistance of trees to the weevil: forced-attack studies and observations of natural infestations in the nursery. In the forced-attack studies, seedlings to be tested are caged (fig. 1) with either weevils or infested material from which weevils will soon emerge. The use of infested material has proved most effective. By caging, theoretically an equal opportunity exists for each tree in a test to be attacked, and the factor of chance escape is greatly reduced. However, weevil attacks are often very numerous and trees are killed which may easily survive under fairly high, natural field populations. Likewise, since it is impossible to equalize the population intensity from one test to another, only comparative rather than absolute evaluations may be made from the results.

The other procedure, observing the effect of natural weevil infestations on different pines, is used where practical. The possibility that nonresistant trees will escape attack simply by chance is much greater with this procedure. In natural infestations in the nursery beds at the Institute, the intensity of attack has not been too different from that which may occur in the field.

The layout of the nursery beds in which these studies were conducted was not designed specifically for host resistance tests. Instead the design was for genetical studies of the growth and character of the seedlings. The host resistance studies had to be superimposed on the design for these studies. With but few exceptions, the plants were arranged in the bed with the progeny from a given cross or source planted across the bed and the various combinations in the particular genetic experiment randomly assigned to the rows in the bed (fig. 2). The seedlings were 6 inches apart within rows and the rows were 6 inches apart. Initially there were 10 seedlings in a

[1] Accepted for publication May 16, 1960.

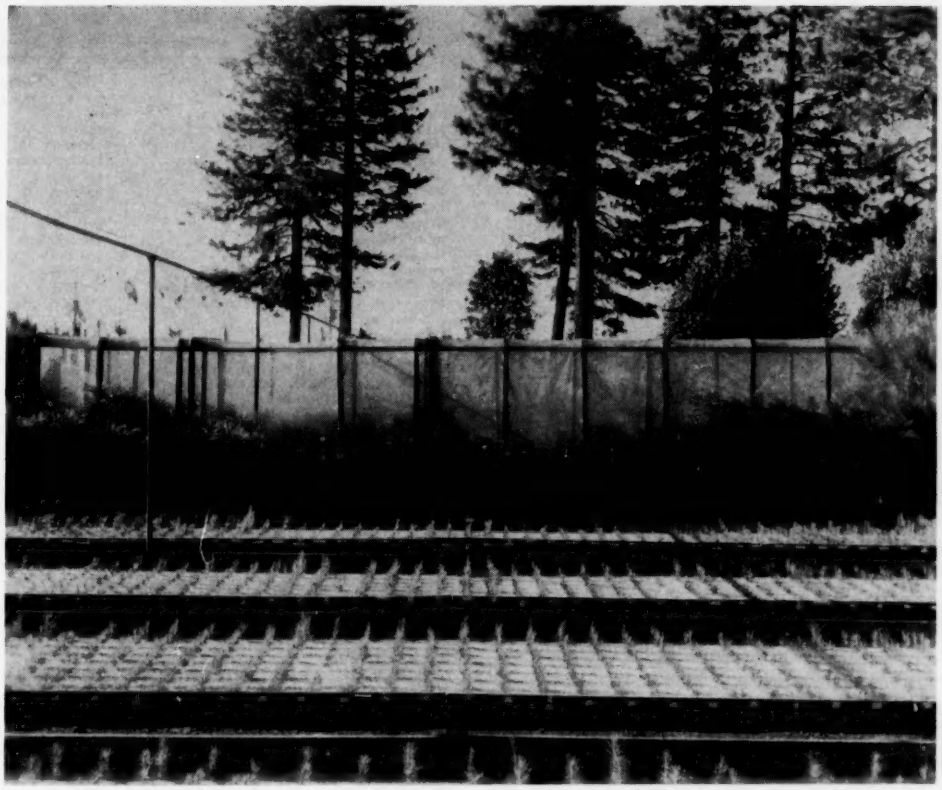

FIG. 1.—Removable cage, over 3-year-old pine seedlings, used to conduct forced-attack tests with the pine reproduction weevil.

row, with as many rows as the design of the genetic experiment demanded or the success of the pollination permitted. After 1 or 2 years in the nursery, some of the seedlings were transplanted to field plots for further genetical studies of growth and character. Therefore the number of trees which were available for weevil-resistance studies varied considerably. Though this layout is easy to establish, maintain, and observe, there are times when it does not seem adequate in overcoming the tendency of the weevil to kill the trees in small groups. A completely randomized distribution might be more suitable for weevil resistance tests.

Most of the studies of weevil resistance in pines have been done with 3- and 4-year-old trees, though a few tests were with 5- and 6-year-olds. Results have been evaluated primarily from the mortality of the various progenies in a test. Sometimes the criterion was frequency of feeding punctures on the needles or number of resin globules resulting from stem punctures. However, an unqualified evaluation was always difficult because of the design of the nursery beds, the apparent variation caused by environmental conditions, and the difficulties in equalizing the intensity of attack between tests.

EARLY STUDIES OF RESISTANCE.—J. M. Miller pursued an intensive 4-year research program involving both the fundamental and applied aspects of resistance of pines to the weevil. His early studies have been summarized (Miller 1950). However, because of the limited distribution of his paper, some of his important results and conclusions will be included in this paper.

In the first series of tests in 1946, Miller compared the mortality of the 6-year-old progeny of Jeffrey pine with mortality of a backcross between Jeffrey pine and a natural hybrid of Jeffrey pine and Coulter pine. This particular hybrid (Jeffrey×(Jeffrey×Coulter)) is of interest to the forest geneticists because Jeffrey pine is a high-elevation, cold-resistant tree, whereas Coulter pine is a drought-tolerant, fast-growing tree. The hybrid might be suitable for reforesting areas in California requiring trees with a combination of these particular characteristics. In the first test the survival under forced-attack conditions was 100% for the hybrid and only 27% for the Jeffrey. The low survival by the latter was expected because Jeffrey pine and ponderosa pine are two of the recognized hosts for the weevil. However, the 100% survival by the hybrid, which was not expected, suggested an association between resistance and Coulter pine. Subsequent tests over the past decade have substantiated this first finding and have shown that a high level of resistance is passed on to various hybrid progeny of the very resistant Coulter pine.

As a result of the early research with this hybrid, tests were established in 1948 to determine its ability to survive under field conditions. The tests were terminated in 1958 and are well documented by Hall (1959). In the field tests the hybrid exhibited superior resistance to the weevil and also excellent resistance to low temperatures. The weevil caused excessive mortality to the Jeffrey pine progeny, and low temperatures killed the Coulter pine progeny. The next practical step has already been taken (Libby

1958) with the establishment of a large-scale pollination program by the U. S. Forest Service to mass-produce this hybrid for planting projects.

The question may be raised concerning the performance of this hybrid over the period of a forest rotation of 100 to 200 years. At the Institute, hybrids of the two parent trees consistently have shown growth and other performance characteristics which are intermediate between those of the two parents. Therefore it may be speculated with a degree of confidence that the hybrid will be suitable for a full stand rotation.

CAUSE OF RESISTANCE.—As part of his early work, Miller attempted to determine why the weevil was able to kill certain trees but unable to kill others. He followed the development of the larva by periodic sectioning and microscopic analysis of attacked stems. He found that in both susceptible and resistant plants, a necrotic area develops around the feeding puncture in the cortex. The reaction of the tree is to wall off the necrotic area from the healthy cortex with a layer of cork cells. In trees which die, the first-instar larva penetrates the necrotic tissue and the healthy cortex and reaches the cambial area. In trees which live, the larvae are rarely able to penetrate the necrotic tissue; the few which do usually die in the cortex.

R. Z. Callaham continued the empirical studies started by Miller and also undertook some research on the possible causes of resistance. Callaham (1960) has analyzed and interpreted much of the data from a genetical standpoint. He concluded that there was considerable intra- and interspecific variation in the level of resistance to weevils in pines and that this level of resistance was apparently passed on to progeny. He also found that there seemed to be a difference in the pattern of attacks, as indicated by needle and stem-feeding punctures, between trees which died and lived.

In an attempt to determine the cause of resistance, Miller's and Callaham's work and notes from subsequent studies have been reviewed. This review brought out several points in accordance with the concepts of Painter (1951) about the ways in which resistance may act.

1. The fact that the young larvae died before reaching the cambial tissue of the trees which survived indicates some toxic action within the plant and suggests Painter's concept of antibiosis.

2. In natural infestations, *i.e.*, not forced attacks, certain species appear to be more heavily attacked and killed than other species. This indicates that nonpreference is one mechanism by which resistance to the weevil operates.

3. Callaham found a significant difference in the number of attacks, as indicated by stem resin globules, on trees which died and on those which lived. Attacks were more numerous on the plants which lived.The reliability of the number of resin globules as an accurate measure of the number of attacks may be questioned because many feeding punctures do not penetrate resin ducts and consequently are not readily visible to the naked eye. Nevertheless, the observations of Callaham indicate that resistance might work through the mechanisms of tolerance or antibiosis.

4. In Miller's early studies and in subsequent observations considerable variation in mortality was found, both within a species and within the progeny of a single tree, which could be attributed to physical factors of the environment.

These points, taken as a whole, support Painter's contention that resistance is seldom the action of one single mechanism but usually the action of two or more.

Table 1.—Pine species and hybrids grouped according to their resistance to the pine reproduction weevil.

NONRESISTANT	RESISTANT
Pinus[a]	Pinus
ponderosa	coulteri
ponderosa var. scopulorum	taeda
ponderosa var. arizonica	lambertiana
jeffreyi	torreyana
banksiana	echinata
murrayana	nigra
sylvestris	nigra var. calabrica
montana	monticola×strobus
washoensis	jeffreyi×coulteri
engelmannii	jeffreyi×(jeffreyi×coulteri)
ponderosa×ponderosa var. scopulorum	[jeffreyi×(jeffreyi×coulteri)] ×ponderosa
ponderosa×ponderosa var. arizonica	
ponderosa×engelmannii	
ponderosa×montezumae	
ponderosa×(engelmannii×montezumae)	
jeffreyi×engelmannii	
engelmannii×montezumae	
murrayana×banksiana	
nigra var. calabrica×thunbergii	

[a] The names and procedure for presenting them follow those used by the Institute of Forest Genetics.

RESISTANT AND NONRESISTANT SPECIES AND HYBRIDS.—One of the continuing lines of research is a screening of pine species and hybrids for resistance to the weevil. Screening is accomplished both by forced-attack studies and by observations of natural infestations. Of the 30 species and hybrids that have been observed, 11 are classed as resistant and 19 nonresistant (table 1). Since no single test included all species and hybrids, the grouping is to some extent arbitrary. In general, 30% or more of the plants of a nonresistant pine were killed in forced-attack studies, and trees in this category were often severely damaged in natural infestations. Plants in the resistant group usually sustained less than 10% mortality in mild forced attacks, and were rarely killed as a result of natural infestations. Under conditions of very severe forced attacks, usually less than 50% of the resistant pines died, whereas mortality in nonresistant ones usually was greater than 90%. The data are not complete enough to warrant a relative rating within the two groups, and no such rating is intended in the arrangement shown in table 1.

Many other pines which have been grown in the nursery at the Institute are not listed in table 1 because of the lack of data. More than likely, they are resistant because the susceptible ones are easily observed and recorded. However, the possibility of chance escape can not be eliminated. Trees listed as resistant in table 1 were either subjected to forced attacks or were growing intimately with plants which were heavily attacked by natural infestations.

Three examples will illustrate why some of the plants were classed as they were. In the first example, data from a severe forced-attack experiment are used (table 2). They show that weevil-caused mortality of wind-pollinated Jeffrey pine was heavy as expected. Mortality of the *jeffreyi×coulteri* hybrid and the three-way hybrid was much less severe. On the basis of these results, the two hybrids are classified as resistant and the Jeffrey pine as

Table 2.—Mortality of 3-year-old pines with severe forced attacks of the pine reproduction weevil.

	TOTAL TREES USED	MORTALITY (%)
Pinus		
jeffreyi×wind	34	91
jeffreyi×coulteri	25	43
(jeffreyi×(jeffreyi×coulteri)) ×ponderosa	38	47
jeffreyi×wind	46	94

nonresistant. It is interesting to note in passing that one-eighth part Coulter pine in the three-way hybrid apparently is sufficient to impart resistance.

The second example is based on data from a natural infestation in a nursery bed of *Pinus nigra* var. *calabrica* and *P. nigra* var. *calabrica*×*thunbergii*, which are schematically presented in figure 2. Mortality from this infestation was 7% for *P. nigra* var. *calabrica* and 68% for the hybrid with *thunbergii*. On the basis of these findings, *P. nigra* var. *calabrica* was placed in the resistant column and *P. nigra* var. *calabrica*×*thunbergii* was placed in the nonresistant column in table 1. Hybrids usually show a resistance intermediate to that of their parents. Although not

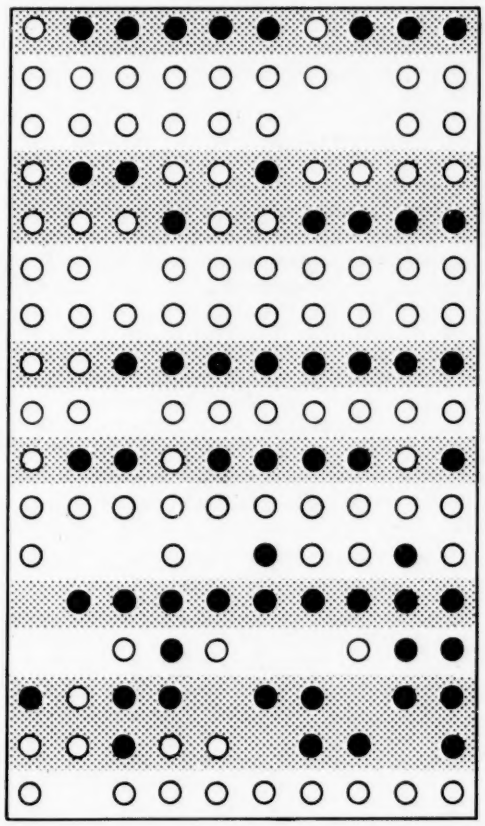

FIG. 2.—Arrangements of pine hybrids and distribution of weevil caused mortality in a typical nursery bed. Living (○) and dead (●) 3-year-old seedlings of *Pinus nigra* var. *calabrica* ×wind (unshaded) and *Pinus nigra* var. *calabrica*×*thunbergii* (shaded).

Table 3.—Mortality of 3-year-old pine seedlings caused by a natural infestation of the pine reproduction weevil.

PLANT COMBINATIONS	TOTAL TREES	MORTALITY (%)
Pinus		
ponderosa×(engelmannii ×montezumae)	32	81
ponderosa×engelmannii	29	97
ponderosa×montezumae	20	70
engelmannii×montezumae	36	92
ponderosa×wind	50	54
murrayana×wind	46	48
murrayana×banksiana	32	53

tested, *P. thunbergii* probably is highly susceptible to the weevil.

The third example is from a natural infestation in a nursery bed of various 3-year-old pines. In this infestation, weevils killed more than 48% of all species and hybrids (table 3). Because of this high mortality, all of these pines were classified as nonresistant.

WITHIN-SPECIES VARIATION IN RESISTANCE.—Several empirical studies were undertaken to determine the cause of within-species variation in resistance. Both ponderosa and Jeffrey pine are, in general, considered nonresistant, but occasionally a tree will be found whose progeny is either much more susceptible or much more resistant than the average. The knowledge of the general level of resistance which a tree imparts to its progeny is of considerable value to the forest geneticist. Wherever possible the more susceptible ones will be avoided and the more resistant ones selected for breeding work.

In one test by Callaham, two ponderosa pines near the Institute of Forest Genetics each were pollinated with five lots of pollen representing four widely separated parts of the extensive range[2] of this pine. The resulting interracial progenies were attacked by the weevil. Pollen source did not influence progeny resistance, but the female parent did have a substantial influence on resistance (table 4). Mortality of progenies of one tree (15–43) did not exceed 10%. In contrast, mortality of the progenies of the other tree (4B-56) exceeded 25%. The test conclusively demonstrated that resistance is heritable and that individuals of ponderosa pine differ significantly in the level of resistance transmitted to their progeny.

[2] Ponderosa pine ranges from British Columbia into Mexico and from Nebraska to the Pacific Coast. In many areas varietal forms are recognized, especially in the southwestern desert regions where the tree occurs in stands that are isolated from the main continuous body of the range.

Table 4.—Per cent weevil-caused mortality to 3- and 4-year-old ponderosa pine progeny of different male and female parents.[a]

	MALE PARENT				
FEMALE PARENT	Cle Elum, Wash.	Monument, Colo.	British Columbia	Crater Lake, Oregon	Grants Pass, Oregon
4B—56	47	30	31	25	32
15—43	0	0	10	10	3

[a] Adapted from Callaham (1960).

Table 5.—Mortality caused by forced and natural attacks of the pine reproduction weevil to 3-year-old pine seedlings of different control- and open-pollinated crosses.

Test No.	Parents[a] (maternal × paternal)	No. Plants Observed	Progeny Mortality (%)	Parent Which Differed	X^2[b]	P[c]
1	J-V21×Ap-V7	6	67			
	J-V21×Ap-V8	9	50	Male	0.00	100
2	P-Eld 4B-65×P-Eld 4B-61	29	24			
	P-Eld 4B-65×P-Eld 4B-66	28	18			
	P-Eld 4B-65×wind	28	21	Male	.43	80
	P-Eld 4B-65×self	22	91			
3	P-Eld 4A-58×P-Eld 4B-65	43	70			
	P-Eld 4A-58×P-Eld 4B-61	27	63	Male	.07	79
4	P-Eld 4B-62×P-Eld 4A-58	32	25			
	P-Eld 4B-62×wind	29	34	Male	.08	78
	P-Eld 4B-62×self	26	77			
5	J-Eld 28-1×J-Eld 27-1	97	8			
	J-Eld 28-1×J-Eld 22-2	97	4	Male	.84	40
	J-Eld 28-1×self	91	23			
6	J-Eld 22-2×J-Eld 28-1	76	38			
	J-Eld 22-2×J-Eld 21-1	77	44			
	J-Eld 22-2×J-Eld 27-1	72	29			
	J-Eld 22-2×wind	77	40	Male	3.73	30
7	P-Eld 4B-62×P-V 294	38	8			
	P-Eld 4B-65×P-V 294	37	19	Female	1.04	30
8	J-Eld 26-1×J.Cl-SB-12	35	21			
	J-Eld 26-3×J.Cl-SB-12	39	3			
	J-Eld 26-4×J.Cl-SB-12	38	7	Female	8.62	2

[a] P=ponderosa, J=Jeffrey, Ap=Apache, J.Cl.=Jeffrey×Coulter, wind=wind pollinated, Eld.=Eldorado, SB=San Benito.

[b] Yates correction used for all 2×2 contingency tables; data from selfing not included in the analysis.

[c] P=probability of obtaining the same X^2 by chance.

Additional data are available to substantiate the viewpoint that resistance to weevils in ponderosa and Jeffrey pine is heritable (table 5). There is also some indication in these data that the female parent may play a greater role than the male parent in the transmission of resistance. These mortality records are from six forced-attack tests and two natural infestations. The data represent progeny of nine different female and 12 different male parents plus wind- and self-pollinated crosses. In the first six tests, the male parent differed, and in the last two the female parent differed.

It was readily apparent that selfing decreased the resistance of a tree's progeny. In test two, for example, mortality was 91% for progeny resulting from selfing and only about 20% for nonselfed crosses. Similarly in test four, there was 77% mortality for progeny from selfing and only about 30% for progeny from nonselfing.

A Chi-square analysis was made of the data, omitting from consideration the progeny of selfing. The Chi-square values and the probabilities of obtaining similar values by random chance are given in the last two columns of table 5. The analysis indicates that the probabilities of chance occurrence were quite high when the male parent differed but quite low when the female parent differed. Further experimentation will be necessary to substantiate or refute this rather tentative conclusion.

Considerable speculation could be set forth to explain this possible maternal influence. Two explanations are as follows: (1) The germinating embryo of a pine seed derives its first energy requirements from the endosperm, the haploid female gametophyte, which is solely the product of the female parent. Therefore, there may be a vigor factor dependent on the food value of the endosperm. A small acceleration in root or stem growth by a seedling during the first few weeks might give the seedling the impetus which enables it to resist weevil attack more readily for the next 2 to 4 years. (2) During the period of germination, hormones and auxins from the endosperm move into the growing sporophyte. Such materials, which could be responsible for resistance, are again solely the product of the female parent.

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Internal DDE Production by Normal and DDT-Resistant Larvae of *Aedes aegypti*[1]

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Abstract

Larvae of 3 DDT-susceptible strains of *Aedes aegypti* (L.) when exposed to 1 p.p.m. DDT for 24 hours, contained approximately one-tenth to one-quarter as much DDE as larvae of three DDT-resistant strains. However, in the naturally-tolerant Penang strain in which the DDT-susceptibility levels had been varied by selection or relaxation of pressure, there was little correlation between DDT-resistance and DDE content.

The possibility that detoxification to DDE might be a mechanism accompanying DDT-resistance in mosquitoes was first investigated by Brown & Perry (1956), who discovered that the larvae of DDT-resistant *Aedes aegypti* (L.) and *A. taeniorhynchus* (Wied.) produced much DDE from high dosages of DDT, while normal larvae of these species produced insignificant amounts of DDE at the much lower dosages they could withstand. Hoskins *et al.* (1958) found that whereas larvae of normal *Culex quinquefasciatus* Say produced a certain amount of DDE, resistant larvae almost completely metabolized the DDT dose to DDE. Bami *et al.* (1957) reported that resistant adults of this species (*C. fatigans* Wied.) produced more DDE than normal adults. Recently Perry (1960) found that adults of a DDT-resistant Turkish strain of *Anopheles sacharovi* Favre produced large amounts of DDE at high doses of DDT, although the DDE/DDT ratio was no greater than that for a slightly-tolerant Greek strain at much lower doses; moreover a resistant laboratory strain of *A. stephensi* Liston produced little more DDE than the normal. Frontali & Carta (1959) also reported that adults of a DDT-resistant strain of *A. atroparvus* van Thiel developed by laboratory pressure on the adults produced twice as much DDE as the normal strain, although a second resistant strain developed by larval pressure still produced the normal amount.

In the present investigation the larval DDE production of three susceptible strains of *Aedes aegypti* is compared with that of three DDT-resistant strains at equivalent doses of DDT. Particular attention has been paid to a normal Malayan strain, a subcolony of which was submitted to laboratory selection pressure, and whose increase in DDT-resistance is described in greater detail by Abedi & Brown (1960).

Material.—The following strains were reared on a larval diet of 9 parts brewer's yeast powder and 1 part blood albumin, the water being treated with 10 p.p.m. streptomycin:

1. Guelph, susceptible, derived from a single female of the Orlando strain
2. N.I.H., susceptible, obtained from the National Institutes of Health
3. Trinidad, susceptible, a mutant-type (*blt,y*) isolate of the above strain
4. Isla Verde, DDT- and dieldrin-resistant, originated from Puerto Rico
5. Penang, susceptible, originated from Penang Island, Malaya
6. Penang, DDT-resistant, developed by laboratory pressure from above strain
7. Malathion-resistant, developed by malathion pressure from Penang strain

The susceptibility levels were assessed by the standard WHO method for mosquito larvae (Brown 1958).

Methods.—Larvae in the late third and early fourth stadia were exposed to 1 p.p.m. DDT for 24 hours at 70° F. in enamel pans; the DDT was added in 5 ml. ethanol to 1,250 ml. distilled water, and 100 larvae were introduced. After exposure they were transferred by a screen spoon to a beaker containing 150 ml. water, thence removed and rinsed with 100 ml. water, and dried on absorbent paper. They were ground to a dry powder in a mortar with sea-sand and anhydrous Na_2SO_4, and extracted three times with a total of 50 ml. CCl_4, which was poured through a column of adsorption alumina (Fisher) 6 cm. long and 13 mm. in diameter.

The purified CCl_4 extract was evaporated to dryness and nitrated with 5 ml. nitrating mixture for 3 hours on a steam bath. The product was dissolved in water, partitioned into ether, treated with NaOH and NaCl, and the ether evaporated, according to the method of Schechter *et al.* (1945). Color was developed by dissolving in 2 ml. benzene and adding 4 ml. sodium methylate. Intensities were measured at 520 and 600 millimicrons wavelength in a Beckman DU spectrophotometer, and the concentrations of DDE and DDT were calculated by the method for two-component systems.

Results.—There was considerable variance in DDE production between successive samples within a strain (table 1). The susceptible Guelph strain produced one-quarter as much DDE as the resistant Trinidad strain, while taking up two-thirds as much DDT. The Penang strain in its normal state produced two-thirds as much DDE as the Trinidad strain. When its LC_{50} had been raised 45 times by laboratory selection pressure with DDT, the F_4 selected generation showed only slightly higher DDE production. This production rate did not decrease, but rather increased, in successive generations which were reverting to susceptibility in DDT-free conditions. That the DDE production in the F_6, F_7 and F_8 relaxed generations was greater than the normal was significant at the 5% level of probability.

The assays were repeated by exposing 25 larvae in 250 ml. water in glass jars (table 2) to the 24-hour 1-p.p.m. dose. The results were similar, the susceptible Guelph strain producing one-ninth as much DDE as the resistant Trinidad strain, and the very resistant F_9 selected generation producing only slightly more DDE than the consid-

[1] Accepted for publication May 18, 1960.

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Table 1.—DDE and DDT content in micrograms per 100 larvae exposed to 1 p.p.m. DDT for 24 hours in 1,250 ml. water in enamel pans.[a]

STRAIN	LC_{50} P.P.M. DDT	NO. OF SAMPLES	DDT	DDE
Guelph susceptible	0.005	11	24.5±3.8	2.3±0.5
Trinidad resistant	1.5	12	34.1±3.9	8.2±1.8
Penang normal	0.08	11	27.5±4.5	5.7±1.0
F_4 relaxed	0.09	7	36.1±7.2	5.5±0.7
F_4 selected[b]	3.6	6	23.2±4.4	6.4±1.1
F_6 relaxed	1.19	9	35.0±5.7	9.9±1.6
F_7 relaxed	0.47	6	20.1±3.1	11.1±1.5
F_8 relaxed	0.26	6	46.2±7.6	10.7±2.0

[a] Averages of 6 to 12 determinations, with standard errors.
[b] Progeny of parents exposed to DDT.

erable production characteristic of the normal Penang strain.

In order to avoid the rather high amounts of DDT remaining with the larvae, the assays were repeated in enamel pans, but this time the larvae were transferred and held for 20 minutes in clean water before assay (table 3). The results were similar, the Guelph strain producing one-ninth as much DDE as the Trinidad strain, while the resistant F_4 and highly resistant F_{11} generations produced only 50% more than the original Penang strain. On the other hand, the DDT- and dieldrin-resistant Isla Verde strain produced 10 times as much DDE as the *blt,y* susceptible strain originating from the Trinidad stock. Four additional strains were tested. Two of these, the N.I.H. Susceptible and the Trinidad Susceptible (*blt,y*) produced little more DDE than the Guelph strain. The other two strains were DDT-resistant, namely the Isla Verde which was also dieldrin-resistant, and the Malathion-resistant in which the developed malathion-tolerance carried a high cross-resistance to DDT; in both of these the DDE production was approximately as high as in the Trinidad Resistant strain.

DISCUSSION.—When the three susceptible strains (Guelph, *blt,y* and N.I.H.) are compared with the three resistant strains (Trinidad, Isla Verde and Malathion-R), it is apparent that the DDE production of the susceptible strains (being 0.9 to 1.9 micrograms) is about one-sixth that of the resistant strains (6.6 to 9.7 micrograms). But it must be remembered that at the concentration of 1 p.p.m. the susceptible strains were knocked down in 1 hour and showed 100% mortality in 6 to 10 hours, whereas the three resistant strains showed respectively 20%, 40% and 5% mortality[3] in the 24-hour exposure period; thus the DDE production was roughly proportional to the duration and amount of survival.

Table 2.—DDE and DDT content in micrograms per 100 larvae exposed in 250 ml. water in glass jars.[a]

STRAIN	LC_{50}, P.P.M. DDT	DDT	DDE
Guelph	0.005	15.0±4.4	0.8±0.6
Trinidad	1.5	9.0±1.8	7.2±0
Penang Normal	0.08	11.2±2.0	7.8±0.7
F_9 selected	18.0	6.2±0.1	8.6±1.0

[a] Calculated from average of two determinations with 25 larvae each.

Table 3.—DDE and DDT content in micrograms per 100 larvae exposed in enamel pans, but subsequently held in clean water for 20 minutes.[a]

STRAIN	LC_{50}, P.P.M. DDT	DDT	DDE
Guelph susceptible	0.005	3.2±1.3	0.9±0
Trinidad susceptible[b]	0.009	7.8±0.9	1.0±0.3
N.I.H. susceptible[b]	0.03	6.0±0.7	1.9±0.2
Penang normal	0.08	6.0±0.3	4.6±0.4
F_4 selected[c]	1.2	19.5±3.3	6.7±0.7
F_{11} selected	40.0	1.5±0.2	6.9±0.5
Trinidad resistant	1.5	9.5±0.9	7.7±0.4
Isla Verde resistant	1.05	15.6±2.2	9.7±2.6
Malathion-resistant	2.6	25.4±1.3	6.6±0.4

[a] Average of two determinations unless otherwise specified.
[b] Average of four determinations.
[c] Average of seven determinations.

In the Penang strain, the DDT-resistant F_4 selected generation produced little more DDE than the parental generation, despite the fact that it showed only 40% mortality instead of the parental 98% mortality level at the test exposure. Moreover the F_6, F_7 and F_8 generations, which were more DDT-susceptible due to relaxation of pressure, and which showed 60% to 90% mortality at the test exposures, produced even more DDE than the DDT-resistant F_4 selected generation.

Whether the lower DDE production of susceptible strains is due to dying sooner can be resolved perhaps by assaying them for the dehydrochlorinating enzyme. Attempts to obtain DDT-dehydrochlorinase activity *in vitro* from resistant *Aedes aegypti* larvae by the method effective for house flies had met with failure (Brown 1956). The method involving homogenization in glutathione, which had succeeded in obtaining this enzymic activity in *Epilachna varivestis* Muls. (Chattoraj & Kearns 1958), was attempted, but it also resulted in failure. Until a method is obtained to assay dehydrochlorinating activity independent of the living organism, it will be difficult to investigate the question of a causative relationship between DDE production and DDT-resistance in mosquitoes.

The results obtained are however sufficient to indicate that the response of the Penang strain to DDT is qualitatively different from that shown by other normal strains. With an initial DDT-tolerance some 10 times greater than the usual, the Penang strain showed a DDE production intermediate between that of the typical susceptible and of the resistant strains. While it rapidly developed DDT-resistance under selection pressure, lost it on relaxation of pressure, the Penang strain did not correspondingly increase or decrease its rate of DDE production to conform with the changes in DDT-susceptibility levels. The DDT-resistance in the Penang strain may thus be suspected to derive from causes other than DDE production. Future studies will therefore investigate the excretion of DDT and DDE as well as the internal production of DDE.

ACKNOWLEDGMENTS.—The authors are deeply indebted to Dr. Z. H. Abedi for assistance in completing the chemi-

[3] The mortality rate in the enamel pans, with 1,250 ml. water, was significantly lower than in the glass jars, with 250 ml. water; such results for *A. aegypti* agree with those reported for *A. taeniorhynchus* by Weidhaas & Gahan (1958).

cal assays. They would like to record their thanks to the following colleagues: Dr. C. C. Hassett, Army Chemical Center, Md., for stock of the Penang and Trinidad strains; Dr. G. B. Craig, University of Notre Dame, for stock of the mutant Trinidad strain; Dr. P. A. Woke, National Institutes of Health, for stock of the N.I.H. strain; Mr. L. Burgess, Canada Department of Agriculture, Guelph, Ont., for stock of the Guelph strain; and Dr. C. W. Kearns, University of Illinois, for a sample of DDE. This work was performed on a grant-in-aid from the National Institutes of Health.

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Control of Several Early-Season Cotton Pests with Insecticides[1]

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ABSTRACT

A mixture of toxaphene plus DDT and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) or methyl parathion gave much better control of the boll weevil (*Anthonomus grandis* Boh.) and the cotton fleahopper (*Psallus seriatus* (Reut.)) than dieldrin or toxaphene. Toxaphene was more effective than Strobane® (mixture of chlorinated terpenes with about 66% of chlorine) against thrips (*Frankliniella* sp.), but both materials gave poor control of cotton fleahoppers and boll weevils. In mixtures with DDT the materials were equally effective against these pests. Sevin® (1-naphthyl methylcarbamate) in a wettable-powder formulation was effective against thrips, cotton fleahoppers, and boll weevils, but rains occurring a short time after application reduced its effectiveness. Shell SD 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), Shell SD 3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate), Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate), and dimethoate were as effective as dieldrin in thrips control.

Experiments were conducted in central Texas in 1959 to evaluate new insecticides against several early-season cotton insects and to obtain additional data on increased tolerance to some of the chlorinated hydrocarbons in populations of the cotton fleahopper (*Psallus seriatus* (Reut.)) and overwintered boll weevils (*Anthonomus grandis* Boh.) which became evident in 1958 (Parencia & Cowan 1960). The only other early-season pest of importance was thrips (*Frankliniella* sp.). Infestations of the boll weevil, the bollworm (*Heliothis zea* (Boddie)), and to a lesser degree, the desert spider mite (*Tetranychus desertorum* Banks) were factors later in the season.

FIELD-CAGE TESTS AGAINST OVERWINTERED BOLL WEEVIL.—Three experiments were conducted with several insecticides against overwintered boll weevils. Immediately after an insecticide was applied, plants were inspected and 1-pint ice-cream cartons were placed over the terminal buds in which weevils were found. Usually the weevils were caged individually, although two were sometimes placed in one cage if they happened to be in the same terminal bud. The number caged per treatment ranged from 20 to 30. Weevils were not handled, but their movement was restricted to the terminal buds on which they were caged.

The cotton in these tests was planted several weeks before other cotton in the vicinity and started fruiting earlier, about June 1. Because of the earlier fruiting overwintered weevils concentrated in the field. Plant inspections indicated a population of 900 weevils per acre prior to the tests.

The performance of Sevin® (1-naphthyl methylcarbamate) in test 1 was probably influenced by a heavy rain within 24 hours after treatment. The formulation, a wettable powder, was apparently washed off the plants. The average kills obtained with the insecticides used in tests 1 and 2 were as follows: Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) 100%, toxaphene plus DDT 87.2%, Sevin 87.1%, toxaphene 67.1%, and dieldrin 55.4% (table 1). Thus the overwintered populations showed considerable tolerance to the last two insecticides.

[1] Accepted for publication May 20, 1960. Presented at the meeting of the Southwestern Branch, Entomological Society of America, El Paso, Texas, February 8–9, 1960.

[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Kill of overwintered boll weevils with several insecticides.

Insecticide (lb.)		No. of Weevils Caged	Per Cent Kill After Days Shown			
Per Gallon	Per Acre		1	3	5	6
Test 1 (Treated on June 3)[a]						
Dieldrin 1.5	0.25	25	—	—	66.7	69.5
Sevin[b]	1.0	25	—	—	75.0	79.2
Guthion 1.5	.25	25	—	—	100.0	—
Toxaphene 6	2.25	25	—	—	50.0	50.0
Toxaphene 4+DDT 2	1.5+.75	30	—	—	85.7	89.3
Untreated	—	27	—	—	—	—
Test 2 (Treated on June 9)[c]						
Dieldrin 1.5	0.25	20	5.0	36.8	—	41.2
Sevin[b]	1.0	20	45.0	95.0	—	95.0
Guthion 1.5	.25	20	95.0	100.0	—	—
Toxaphene 6	2.25	20	15.8	84.2	—	84.2
Toxaphene 4+DDT 2	1.5+0.75	20	70.0	85.0	—	85.0
Untreated	—	20	—	—	—	—
Test 3 (Treated on June 9)[c]						
Sevin mull 2.5	1.25	27	36.0	62.5	—	69.6
Sevin[b]	1.25	27	40.7	75.0	—	78.3
Methyl parathion 2	0.5	24	95.6	100.0	—	—
Toxaphene 4+DDT 2	1.5+0.75	25	41.7	87.0	—	90.9
Untreated	—	27	—	—	—	—

[a] 2 inches of rain within 24 hours, 1 inch on third day.
[b] Wettable powder, 85% active ingredient.
[c] 1½ inches rain on third day.

In test 3, 1.5 inches of rain fell on the third day after treatment. Methyl parathion gave 100% kill. A high kill was obtained with toxaphene plus DDT. Sevin was less effective, the wettable powder being more effective than a mull formulation.

FIELD EXPERIMENTS AGAINST SEVERAL COTTON INSECTS.—Six experiments were conducted with various insecticides. In experiments 1 and 2 the plots were 16 rows wide and of sufficient length to make 0.2 acre. They were arranged in randomized blocks with 4 replications. Untreated checks were not included within the experimental design, but were located in the same field adjacent to the treated plots. In experiment 3 the plots were 32 to 48 rows wide and 2 to 5 acres in size with three replications. Treatments were not replicated in the other experiments.

Methods of applying insecticides early in the season were the same as those reported by Davis *et al.* (1957). Applications were made at about 7-day intervals, although, because of rain, as many as 11 days elapsed between some applications. Late-season applications were made in accordance with those reported by Cowan *et al.* (1956) at approximately 5-day intervals.

The procedures for recording data on the boll weevil, bollworm, and cotton fleahopper were the same as those reported by Ewing & Parencia (1943). Records on thrips were obtained by counting the number on 20 plants selected at random in each plot. Spider mite populations were determined by counting the number per square inch of leaf surface on 100 leaves randomly selected in each plot.

In experiment 1 toxaphene at 1.12 pounds, toxaphene and Strobane® (a mixture of chlorinated terpenes with about 66% of chlorine) each at 0.75 pound plus DDT at 0.37 pound, toxaphene at 1.5 pounds and toxaphene at 1.0 pound plus DDT at 0.5 pound gave significantly better thrips control than Strobane at 1.12 pounds per acre. Cotton fleahopper infestations developed after treatment for thrips control was over. The same materials at higher dosages were used for this control. Dosages of 1.0 pound of toxaphene or Strobane plus 0.5 pound of DDT gave significantly better control than 1.5 pounds of toxaphene, 1.5 pounds of Strobane, or 2.25 pounds of toxaphene (table 2). Similar results were obtained with toxaphene in 1958 (Parencia & Cowan 1960).

For late-season control of the boll weevil and bollworm all the plots were treated with toxaphene-DDT on July 15 and 23 and with toxaphene-DDT-methyl parathion on July 31. Although treated plots showed good increases in yield, there were no significant differences between treatments. Later in the season moisture conditions were favorable for the replacement of fruit lost to fleahoppers earlier in the season. However, yields from treatments that gave poor fleahopper control were lower than those where good control was obtained (table 3).

In experiment 2 dieldrin, Shell SD 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), Shell SD 3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate), Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate), and dimethoate gave significantly better thrips control than Sevin (table 2). Sevin was used in a wettable-powder spray, and

Table 2.—Effectiveness of several insecticides applied as sprays against thrips, cotton fleahoppers and overwintered boll weevils in field plot experiments.

Insecticide (lb.)		Number of		Weevil Punctured Squares[a] (%)
Per Gallon	Per Acre	Thrips Per Plant	Fleahoppers Per 100 Terminals	
Experiment 1 (5 applications between May 15 and June 17)[b]				
Toxaphene 6	1.2	1.4	18.6	5.5
	1.65	1.0	14.5	6.0
Strobane 8	1.2	2.8	16.5	6.2
Strobane 4+DDT 2	0.8 +0.4	1.1	10.0	3.2
Toxaphene 4+DDT 2	.8 + .4	0.7	8.8	5.8
	1.1 + .55	.8	7.7	4.5
L.S.D. at 5% level	—	.9	2.6	—
Untreated (outside check)	—	9.0	21.0	13.0
Experiment 2 (3 applications between May 21 and June 11)				
Dieldrin 1.5	0.1	1.5	8.1	21.6
Sevin[c]	.5	3.9	7.2	21.7
Shell SD 4402 1.25	.25	1.0	7.8	18.7
Shell SD 3562 10	.5	1.2	5.4	20.8
Bayer 25141 2	.25	1.4	8.4	21.8
Dimethoate 4	.25	2.1	8.2	20.8
L.S.D. at 5% level	—	0.9	—	—
Untreated (outside check)	—	10.2	13.1	25.6
Experiment 3 (4 applications between May 19 and June 17)				
Strobane 8	1.81	3.0	23.2	21.1
Toxaphene 6	1.81	2.2	18.5	17.6
Strobane 4+DDT 2	1.21+0.6	1.8	9.0	9.9
Toxaphene 4+DDT 2	1.21+ .6	1.3	7.2	10.5
Methyl parathion 2	0.35	3.6	10.2	7.6
Sevin[c]	.89	3.0	8.2	12.3
Untreated	—	8.6	19.1	30.5
L.S.D. at 5% level	—	1.3	5.6	8.3
Experiment 4 (3 applications between June 3 and 16)				
Dieldrin 1.5	0.25	—	9.3	25.8
Sevin[c]	1.0	—	4.6	3.5
Guthion 1.5	.25	—	6.0	2.2
Toxaphene 6	2.25	—	13.9	7.8
Toxaphene 4+DDT 2	1.5 + .75	—	3.7	8.6
Untreated	—	—	14.2	71.8
Experiment 5 (2 applications June 9 and 16)				
Sevin mull 2.5	1.25	—	6.6	17.0
Sevin[c]	1.25	—	4.3	6.2
Methyl parathion 2	0.5	—	4.5	5.0
Toxaphene 4+DDT 2	1.5 + .75	—	5.5	5.8
Untreated	—	—	11.7	66.0
Experiment 6 (1 application June 18)				
Toxaphene 4+DDT 2	1.0 + .5	—	8.0	3.3
Sevin[c]	1.0	—	8.4	5.3
Untreated	—	—	23.8	23.3

[a] Before late treatment.
[b] Dosages are averages of thrips and cotton fleahopper treatments.
[c] Wettable powder, 85% active ingredient.

Table 3.—Effectiveness of several insecticides applied as aprays against several cotton insects in field plot experiments.

Insecticide (lb.)		Number of		Weevil Punctured Squares (%)	Bollworm-Injured (%)		Seed Cotton (lb./a.)	
Per Gallon	Per Acre	Spider Mites Per Square Inch	Fleahoppers Per 100 Terminals		Squares	Bolls	Total	Gain Over Check
Experiment 1 (5 applications between May 15 and June 17)								
Toxaphene 6	1.2	—	18.6	26.6	3.7	3.5	764	351
	1.65	—	14.5	26.8	3.6	4.2	797	384
Strobane 8	1.2	—	16.5	23.2	3.0	3.2	706	293
Strobane 4+DDT 2	0.8 + .4	—	10.0	24.6	2.6	4.5	846	433
Toxaphene 4+DDT 2	.8 + .4	—	8.8	26.0	3.0	4.5	873	460
	1.1 + .55	—	7.7	26.5	3.3	2.8	871	458
L.S.D. at 5% level	—	—	2.6	—	—	—	—	—
Untreated (outside check)	—	—	21.0	33.7	2.8	4.5	413	—
Experiment 2 (8 applications between July 8 and August 12)								
Dieldrin 1.5+DDT 2	0.5 +1.0	—	8.1	43.5	2.2	4.6	872	567
Sevin[a]	1.25	—	7.2	37.9	2.7	4.3	992	687
Shell SD 4402 1.25	.5	—	7.8	41.4	2.3	4.1	813	508
Shell SD 3562 10	1.0	—	5.4	43.0	6.8	11.4	378	73
Bayer 25141 2	.5	—	8.4	39.9	5.0	8.3	649	344
Toxaphene 4+DDT 2	2.0 +1.0	—	8.2	41.5	2.5	3.9	927	622
L.S.D. at 5% level	—	—	—	—	1.3	2.9	262	—
Untreated (outside check)	—	—	13.1	59.4	6.2	10.4	305	—
Experiment 3 (7 applications between June 30 and August 11)								
Strobane 4+DDT 2 +methyl parathion 2	2.0 +1.0+.25	0	23.2	27.2	2.1	2.7	1,236	851
Toxaphene 4+DDT 2 +methyl parathion 2	2.0 +1.0+.25	.02	18.5	23.6	2.6	2.2	1,237	852
Strobane 4+DDT 2	2.0 +1.0	.44	9.0	20.1	2.2	2.6	1,294	909
Toxaphene 4+DDT 2	2.0 +1.0	.22	7.2	19.1	2.4	2.2	1,435	1,050
Methyl parathion 2+DDT 2	.5 +1.0	0	10.2	16.8	3.2	3.0	1,395	1,010
Sevin[a]	1.25	.89	8.2	18.4	2.8	2.3	1,459	1,074
Untreated	—	0	19.1	54.7	4.0	6.0	385	—
L.S.D. at 5% level	—	—	5.6	9.9	1.0	1.4	242	—
Experiment 4 (8 applications between June 30 and August 3)								
Dieldrin 1.5+DDT 2	0.47+1.0	.78	9.3	54.4	4.5	4.7	1,163	908
Sevin[a]	1.25	.91	4.6	13.9	2.1	3.6	1,735	1,480
Guthion 1.5+DDT 2	.25+1.0	.01	6.0	8.7	3.5	4.6	1,656	1,401
Toxaphene 4+DDT 2	2.0 +1.0	.05	13.9	25.0	3.2	2.2	1,721	1,466
	2.0 +1.0	.39	3.7	24.1	2.5	2.2	1,713	1,458
Untreated	—	0	14.2	73.3	3.6	5.0	255	—
Experiment 5 (7 applications between June 30 and August 3)								
Sevin mull[b] 2.5	1.39	0.91	6.6	47.2	2.5	2.8	1,294	623
Sevin[a,b]	1.25	.50	4.3	31.9	3.7	3.0	1,936	1,265
Methyl parathion 2+DDT 2	0.5 +1.0	.05	4.5	30.7	4.0	3.7	1,784	1,113
Toxaphene 4+DDT 2	2.0 +1.0	1.14	5.5	31.2	2.0	2.6	1,621	950
Untreated	—	.94	11.7	79.5	2.8	3.0	671	—
Experiment 6 (5 applications between July 1 and 28)								
Toxaphene 4+DDT 2	2.0 +1.0	.02	8.0	14.2	3.4	4.3	995	605
Sevin[a]	1.25	.54	8.4	7.8	3.0	4.4	850	460
Untreated	—	0	23.8	48.1	7.9	9.7	390	—

[a] Wettable powder, 85% active ingredient.
[b] 0.25 lb. of demeton added on July 28.

rains within 24 hours apparently reduced its effectiveness. Six days after the first application there was no significant difference between treatments, but after 11 days all the other treatments were significantly better than Sevin. There was no difference between treatments 1 day after the second application was made. Noticeable phytotoxicity occurred in plots treated with dimethoate.

Bollworms and boll weevils became factors late in the season, and the same materials were applied for their control except that toxaphene-DDT was used instead of dimethoate, which is phytotoxic at boll weevil dosages. DDT was added to dieldrin in the plots originally receiving that treatment (table 3). In boll weevil control there was no difference between treatments. Dieldrin-DDT, Sevin, Shell SD 4402, and toxaphene-DDT gave significantly better bollworm control than Shell SD 3562 or Bayer 25141. Shell SD 3562 gave a significantly lower yield than any of the other insecticides, and toxaphene-

DDT and Sevin gave significantly better yields than Bayer 25141.

In experiment 3 toxaphene and Strobane each with DDT gave significantly better thrips control than methyl parathion, and toxaphene-DDT was better than Strobane or Sevin. Rains apparently reduced the effectiveness of the Sevin wettable-powder spray. Methyl parathion gave little residual control of thrips. Toxaphene or Strobane plus DDT, methyl parathion, and Sevin gave significantly better cotton fleahopper control than Strobane or toxaphene alone. Strobane gave poorer control of overwintered weevils than any of the other insecticides except toxaphene. None of the materials afforded good control because of rainy weather during the first 2 weeks in June, either preventing applications from being made on schedule or reducing their effectiveness (table 2).

For late-season insect control DDT and methyl parathion were added to the Strobane and toxaphene treatments used earlier and DDT was added to methyl parathion. The dosages of all treatments were increased (table 3). Each insecticide reduced the boll weevil infestation significantly below the check, and methyl parathion-DDT was better than Strobane-DDT-methyl parathion. However, fruiting had been delayed by cotton fleahoppers in the plots receiving Strobane and toxaphene. There was slightly more bollworm injury in the methyl parathion plots during the time between early and late applications than in the plots of the other insecticides.

Plants in the plots receiving toxaphene or Strobane plus DDT set fruit and matured earlier than in those receiving methyl parathion plus DDT. Maturity in the Sevin plots was intermediate to that of the toxaphene or Strobane plus DDT and methyl parathion-DDT plots. Plants in the methyl parathion-DDT plots matured at about the same time as those that had originally received Strobane and toxaphene. In the Strobane and toxaphene plots fruiting had been delayed by cotton fleahoppers. It appeared that the poorer control of thrips and greater bollworm injury during midseason influenced the delay in maturity in the plots originally receiving methyl parathion. However, other factors may have been involved, since plant maturity approximated that in the plots receiving toxaphene or Strobane even though these insecticides gave poorer fleahopper control than methyl parathion.

Each insecticide produced a significant increase in yield over the untreated check. The yield in the check was very low. Rains late in July were favorable for the production of late fruit, which when protected by late treatment replaced fruit lost early in the season. However, yields were lowest in the plots originally receiving toxaphene and Strobane.

In experiment 4 toxaphene and dieldrin gave the poorest control of a light cotton fleahopper infestation and dieldrin gave the poorest control of overwintered weevils (table 2).

For late-season control the plots receiving dieldrin, Guthion, and toxaphene received mixtures of the respective insecticides with DDT. As in overwintered weevil control, dieldrin-DDT gave poor control of the boll weevil later in the season. Sevin and Guthion-DDT gave good weevil control and toxaphene-DDT was only slightly less effective. There was some noneconomic desert spider mite buildup in the plots treated with Sevin, dieldrin-DDT, and to a lesser degree with toxaphene-DDT. In bollworm control there was little difference between treatments. The yield in the check was only 255 pounds of seed cotton per acre. Gains from the Sevin, Guthion-DDT, and the two toxaphene-DDT treatments ranged from 1,401 to 1,480 pounds per acre. Dieldrin plus DDT produced a gain of only 908 pounds, reflecting the poorer boll weevil control obtained with it (table 3).

In experiment 5 the poorest overwintered weevil control was obtained with the Sevin mull formulation (table 2). Bollworm square injury following use of methyl parathion early in the season was greater than in the other treatments.

For late-season insect control DDT was added to methyl parathion and dosages of other materials were increased. The Sevin mull gave poorer weevil control than the wettable-powder spray, and emulsion sprays of methyl parathion-DDT or toxaphene-DDT (table 3). Desert spider mite infestations built up in the plots receiving both Sevin formulations, and demeton was included for their control in the application made on July 28. Each treatment gave good bollworm control. Good increases in yield were produced by the insecticides, but the increase from the Sevin mull was considerably less than from the other treatments, reflecting the poorer boll weevil control obtained with it.

In experiment 6 there was no difference in cotton fleahopper control between toxaphene-DDT and Sevin (table 2). The plots received the same insecticides at higher dosages for boll weevil and bollworm control later in the season (table 3). Both Sevin and toxaphene-DDT gave good control of these pests. Desert spider mite infestations were greater following use of Sevin than following toxaphene-DDT. Both treatments gave good increases in yield over untreated check.

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Interference with the Biological Control of Cottony-Cushion Scale by Insecticides and Attempts to Re-establish a Favorable Natural Balance[1]

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Abstract

Biological control of the cottony-cushion scale, *Icerya purchasi* Mask., by the vedalia beetle, *Rodolia cardinalis* (Muls.), in the desert citrus areas of California was upset so severely by insecticidal drift from surrounding treated areas that chemical control became necessary. Although, in the desert, vedalia activity is restricted from November to March by cold temperatures, *Icerya* is normally controlled biologically before June unless insecticidal drift interferes with vedalia. Vedalia destruction is most often caused by drift of malathion, parathion, or DDT applied by airplane during the early spring months. Malathion drift is worse than parathion because it produces no balancing destruction of the host. Drift residues from multiple treatments are toxic for long periods. The problem of *Icerya* on desert citrus was largely eliminated by restricting drift. When vedalia could be obtained, its colonization controlled *Icerya* outbreaks. Vedalia propagation was found to be impractical, but field-collected pupae or adults may be stored for long periods. Periodic colonization of *Cryptochaetum iceryae* (Will.) was tested, but considered impractical. Other natural enemies introduced against *Icerya* were not successfully established.

There has been a long history of insecticide-caused upsets in the biological control of cottony-cushion scale. Brann (1919) first reported the upset by lead arsenate of *Icerya purchasi* Mask. in California citrus areas and correlated the outbreaks with the destruction of the vedalia, *Rodolia cardinalis* (Muls.). Nel (1942) recorded similar upsets in the biological control of the scale insect on pears in South Africa when vedalia was destroyed by lead arsenate. DeBach (1946) reported on outbreaks of cottony-cushion scale associated with the destruction of vedalia by rotenone. Woglum (1946) first noted the tendency of DDT to provoke cottony-cushion scale by killing vedalia, and DeBach (1947) further considered the destruction of vedalia by DDT in relation to increase of the scale. Carman *et al.* (1954) noted increased *Icerya* infestations on citrus in central California induced by malathion drift from adjoining treated properties and tentatively associated the increase with a possible repellent action of malathion on vedalia.

In the Coachella and Imperial desert valleys of California, cottony-cushion scale had been considered under complete biological control ever since the importation of vedalia in 1888–89. However, in the early 1950's, the scale insect became, for reasons then unknown, increasingly serious as a pest of citrus in these regions. By 1956 a large part of the citrus acreage was threatened with annual infestations of the pest. The problem became so widespread that it was necessary to develop a standard recommendation for its treatment. The current California citrus spray program now contains recommendations for the use of relatively high dosages of malathion and of parathion as protective measures against this scale insect.

Starting in 1956 a field survey was undertaken to determine (1) why and how the normal biological control of cottony-cushion scale was being disrupted, and (2) the means by which it might be re-established. The survey included all areas of reported outbreaks of cottony-cushion scale in the Coachella and Imperial valleys of California and some of the areas of infestation that had developed under comparable desert-citrus conditions in Arizona. Developing beyond the original design, the survey included also an examination of the activity patterns of vedalia in the field and of various ways in which the natural enemies of cottony-cushion scale may be manipulated to correct outbreaks of the pest.

The Normal Pattern of Cottony-Cushion Scale Control by Vedalia.—Examination showed that *Rodolia cardinalis* was the only natural enemy present on cottony-cushion scale under desert conditions. Except for favored locations such as *Icerya*-infested ornamentals near heated buildings, the vedalia normally disappears from citrus in the desert area from the onset of cold weather in late November until the warm periods of late February and of March. The period of disappearance of vedalia from the orchards may be prolonged by colder temperatures or more extended winter conditions. During exceptionally mild winters, small numbers of both larval and adult predators may survive within the orchards, but their development is greatly retarded. These observations are in accord with previous findings in the central-valley citrus areas where nearly complete absence of vedalia has been observed following long, cold winters and also with the observations of Geier & Baggiolini (1950) on the responses of vedalia to cold temperatures in Switzerland.

During the winter months in the desert, when vedalia is inactive, cottony-cushion scale continues to develop slowly. With warmer weather in late February and March, vedalia activity recommences, and, ordinarily, by late May or June the beetles have completely destroyed all observable host scale insects. After depletion of the host, the adult vedalia make a complete exodus from the grove. One week there may be an abundance of pupae with many adults emerging; the next week, emergence having been completed, not a single adult or larva may be found in the orchard.

Despite appearances to the contrary, however, complete elimination of the *Icerya* has not occurred. Invariably some eggs of the scale remain and they hatch and develop to produce another somewhat lighter infestation of the scale during the following fall and winter. Again the next spring, vedalia causes seemingly complete elimination of the host. In this manner, heavy cottony-cushion

[1] Paper No. 1223, University of California Citrus Experiment Station, Riverside, California. Accepted for publication May 31, 1960.

scale infestations may show a steadily decreasing intensity each season with from 2 to 3 years required for final reduction of the infestation to a stable subeconomic level.

Where the biological control of cottony-cushion scale has been seriously disrupted by long, cold winters which produce nearly area-wide elimination of vedalia, growers have come to rely upon spring inoculation of their orchards with adult vedalia when sources are available from other, warmer areas of the state. Twenty adult vedalia, 10 of which are mated females, are ordinarily sufficient to start a colony. Surprisingly, from such insignificant numbers established in an infested orchard in the spring, complete elimination of the pest can ordinarily be obtained within 3 months, and often within 2 to 2½ months, providing there is no further interference with their normal increase by cold weather or insecticides.

HOW INSECTICIDES INDUCE UPSETS OF COTTONY-CUSHION SCALE.—It was clearly evident from the survey that all of the upsets in the biological control of cottony-cushion scale in the desert areas during 1956 and 1957 were caused by interference with the normal activity of vedalia by insecticidal-drift contaminants arising from treatments of other crops adjoining the citrus acreages. The patterns were in all cases similar. In the majority of the cases examined, a distinct gradation in the cottony-cushion scale infestation was apparent, with the intensity of the infestation increasing toward the adjoining treated-crop areas. These crop treatments are listed in decreasing order of their frequency as causes of cottony-cushion scale upsets: (1) vineyards treated with malathion, (2) vegetables treated with malathion, (3) alfalfa treated with parathion, (4) cotton treated with DDT, and (5) vegetables treated with DDT.

The most significant associations revealed by the survey were three: (1) Malathion was the material most often responsible for local upsets in control of cottony-cushion scale on neighboring citrus. (This, in part, was caused by the frequency of its use on adjoining crops.) (2) Most upsets were associated with airplane applications of dust formulations. (3) Drift contamination in citrus orchards in late April, May, and early June was most often responsible for serious cottony-cushion scale outbreaks upon desert citrus.

Vedalia destruction by insecticidal-drift deposits adhering to citrus foliage caused all the upsets examined; however, there were variations in the way in which the insecticides restricted the activity of the predators as well as in the degree of restriction. Particularly interesting was the general destruction of vedalia which occurred over a widespread area of the Imperial Valley in May, 1957. This area, widely cropped to alfalfa, was usually treated with parathion at least once during May for serious infestations of the spotted alfalfa aphid, *Therioaphis maculata* (Buck.). The general treatment nearly eliminated vedalia from the entire area, leaving only rare small colonies in the orchards most isolated from alfalfa acreages. By that time of the year various degrees of biological control of the host had been attained so that in a few cases the scale had been eliminated before the suppression of vedalia, and in other cases partial control existed.

In the Imperial Valley area, and in other areas where a comparison could be made of the effects against vedalia of parathion and of malathion, a basic difference in the action of the two insecticides was illustrated. When dosages were similar, the two materials had similar effects on the vedalia but dissimilar effects on the *Icerya*. The parathion drift produced a balancing kill of the small stages of the scale; malathion did not. This difference, intensified by the heavier dosages of malathion used in the field and the resulting increase in persistence of toxic residues, eventually produced much greater upsets from the malathion drift than from the parathion drift.

In the laboratory, comparative tests against adult vedalia were run using 50 materials. These tests showed that malathion was only slightly more rapid in its action against vedalia than parathion at similar dosages. Among the materials considered highly toxic to vedalia at common field dosages, malathion was followed in decreasing order of toxicity by rotenone, parathion, BHC, Dilan® (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane), Chlorthion® (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate), Diazinon® (*O-O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), methoxychlor, endrin, DDT, and calcium arsenate.

The data from the field survey also revealed that the most complete elimination of vedalia occurred through a series of drift deposits from multiple treatments of the adjoining crops. Reliable information on neighboring treated crops was often difficult to obtain, but it appeared that, in general, a single drift deposit might hold vedalia activity static for a few weeks and act only as a temporary deterrent to the predator. Two deposits with 3 or fewer weeks intervening were much more disastrous than would ordinarily have been expected.

Citrus leaves exposed to drift contamination from two or three treatments of adjoining crops with malathion dust were bioassayed using vedalia adults. These tests showed that toxic deposits existed at least 1 month after the last treatment of adjoining properties. In one case where lettuce fields neighboring on citrus received winter applications of malathion every 5 days, attempts to establish vedalia were futile during the early spring months because of the unexpected persistence of toxic residues. The toxic action of the materials encountered in the field survey was more drastic against adult than against larval stages of vedalia.

THE PREVENTION OF COTTONY-CUSHION SCALE OUTBREAKS.—A practical solution to the problem of cottony-cushion scale outbreaks on desert citrus in California has been developed primarily through preventive measures. These measures, aimed at avoiding insecticidal-drift contamination, consist of (1) paying proper attention to direction of prevailing winds during application, (2) shifting from airplane to ground application wherever practicable, and (3) using spray rather than dust formulations if possible. Acceptance of these conditions for use of pesticides has been obtained almost exclusively through individual appeal by each citrus grower for cooperation from the owners of adjacent acreages. As a result of this personal cooperation, the severity of cottony-cushion scale outbreaks in the California desert-citrus areas has gradually subsided in the past 2 years to a point where economic infestations are now relatively rare.

OBTAINING VEDALIA FOR CORRECTION OF COTTONY-CUSHION SCALE OUTBREAKS.—During the studies of the

cause and prevention of cottony-cushion scale outbreaks on desert citrus, other possible approaches to corrective action against existing infestations of the pest were examined. The survey had shown that these infestations resulted from the critical interruption of vedalia activity by orchard contamination from drift during late April, May, and early June. It was found by trial that vedalia inoculation in the infested orchards in the late summer and fall months, after the degradation of toxic residues, sometimes provided control of the cottony-cushion scale before the onset of cold weather, but that this method could not always be depended upon. However, when vedalia were inoculated in the very early spring, they usually could be depended upon to destroy the scale before the normal period of exposure to insecticidal drift. This process hinged largely upon the availability of vedalia in the early spring months. To obtain other than very limited quantities of the predator from ornamentals in the warmer coastal areas of the State was virtually impossible. Newell (1919) suggested that vedalia cultures could be maintained in the laboratory by frequent additions of field-collected *Icerya*-infested twigs. This procedure was tested and found to be too time-consuming for the few weak adults obtained. A large number of plants were tested for their suitability as glasshouse hosts for *Icerya purchasi*. Citrus seedlings and the centipede plant, *Homalocladium platycladum*, were found to be most suitable, but heavy infestations of the scale resulted in such rapid destruction of them and all the other plant hosts tested that their use was impractical for the rearing of vedalia. In these trials it was interesting that *Icerya*-infested Spanish broom, *Spartium junceum*, which is supposedly incapable of supporting vedalia (Quayle 1938), was as readily attacked under confinement as were the other test plants.

A system of infesting field-growing *Pittosporum tobira* plants with pure stocks of *Icerya* and covering the plants with DDT-treated wire screen cages to keep out parasites and predators resulted in good infestations of cottony-cushion scale. These plants, when re-covered with a new cloth-covered cage and inoculated with vedalia, provided a rotating program for the continuous production of vedalia. Although some vedalia were produced by this method, the plants rapidly degenerated and the vedalia development was severely restricted in the winter despite the warm location chosen. It was of interest that extraneous adult vedalia frequently found and rested upon the outside of these cages indicating their chemotrophic response to the host.

Trials were undertaken to hold field-collected vedalia in cold storage for survival over the winter months from October, when they could be readily collected in infested orchards, to March, when field recolonization was desired. Field-collected adults and pupae held at temperatures of 35°, 40°, 50°, and 60° F. and 40% and 70% relative humidity (R. H.) demonstrated that at the optimum of 60° F. and 70% R. H. the adults, when removed from storage and fed honey once per week, could be stored for a period of 82 days with 60% survival. Refrigeration failure terminated the trials at that time. An undetermined percentage of the pupae survived for a like period with the optimum storage for this stage at 50° F. and 70% R. H. The successful storage of field-collected pupae for longer periods appears probable. Some individuals which emerged from stored pupae were retained as adults for a combined holding period of 4½ months, and laid viable eggs.

TRIALS WITH OTHER NATURAL ENEMIES OF COTTONY-CUSHION SCALE.—The dipterous parasite *Cryptochaetum iceryae* (Will.), which rivals, if not exceeds, vedalia in effectiveness against cottony-cushion scale (Thorpe 1930) in the coastal areas of southern California, was not found in the desert citrus areas. This species is extraordinarily effective during the winter and early spring months in the California coastal and intermediate inland areas. With its recognized tolerance to low temperatures it was considered for colonization in the desert in the winter periods when vedalia was handicapped by cold weather.

In January, 1957, colonization of the very short-lived adult *Cryptochaetum* was attempted in three desert citrus orchards infested with *Icerya*. Some adult *Cryptochaetum* were confined in organdie sleeve cages on scale-infested branches, and some were released directly upon the infested trees. After 1 month, no evidence of establishment was observed. During February, a further colonization trial was made using *Cryptochaetum*-infested cottony-cushion scale from which a constant emergence was taking place. Establishment was procured through this technique. A heavy increase in the parasite population took place and a high degree of scale control occurred before vedalia, which was released in late March, finally outnumbered the parasite and eliminated the host. The parasite was not recovered from this orchard during the following 2 years, but the scale was so rare that adequate recovery samples could not be obtained.

It appears likely that satisfactory winter control of cottony-cushion scale could be accomplished through the periodic release of *Cryptochaetum iceryae*. However, because of the difficulty in establishing this delicate species and because of its probable seasonal elimination by the high desert temperatures, this procedure is not considered a dependable or practical means of controlling *Icerya purchasi* on desert citrus.

In 1957 a phorid, tentatively identified as *Syneura cocciphila* Coq., was collected by the senior author from *Icerya* sp. in the state of San Luis Potosi, Mexico. But attempts to import this species into California were unsuccessful. Information now at hand indicates that the *Syneura* sp. obtained from Mexico may not be well adapted to *I. purchasi* and may be distinct from *S. cocciphila* (=*infraposita* B. & S. of Autuori (1928)) judging from its distinctive habit of parasitizing immature stages of *Icerya*.

In 1958 *Rodolia foedata* (Muls.) was shipped by the senior author to California from Bulawayo, Southern Rhodesia, and another species identified as *Rodolia iceryae* Janson was sent from Muden, Union of South Africa. They were received as living adults in Riverside. No apparent establishment resulted from field colonization if these species in either southern or northern California. *R. foedata* is known to be active in areas of the Union of South Africa too cold for *R. cardinalis* (Mally 1916), so this species may be useful in California if it can be established. Little is known of the cold tolerance of *R. iceryae* or of the dipterous *Syneura* species.

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Screw-Worm Control with Insecticide Sprays[1]

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ABSTRACT

Experiments were conducted to find a safe and inexpensive insecticide that is toxic to young larvae and has a 2-day residual effect. In laboratory tests Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate) gave excellent control of screw-worms (*Callitroga hominivorax* (Cqrl.)) in sheep. In field trials it performed as well as Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) on sheep but not on cattle. Further critical laboratory tests confirmed the field results.

As early as 1956 Kaplanis and coworkers (1959) found that Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) sprayed on cattle did not function systemically against screw-worms (*Callitroga hominivorax* (Cqrl.)), but that minute amounts of insecticide "drifting" from residues in the hair protected wounds by killing newly hatched larvae. Brundrett & Graham (1958) found that the residual protective effect of 0.25% Co-Ral sprays applied to newly shorn sheep and goats usually persisted for 2 to 3 weeks under Texas field conditions.

In 1958 Graham and coworkers conducted some exploratory laboratory tests spraying artificially infested sheep with a number of phosphorus insecticides. From this group they selected ronnel and Co-Ral for comparative field tests in Texas (Graham *et al.* 1959). As a result of those studies the Entomology Research Division (1959) recommended these materials for the control and prevention of screw-worm infestation.

Besides the usual agricultural recommendation, it was suggested that a 0.5% ronnel or 0.25% Co-Ral spray be used at inspection stations to treat livestock shipped from screw-worm-infested areas into those Southeastern States recently freed of screw-worms. At those stations infested wounds are treated with EQ-335 dressing (Division of Insects Affecting Man and Animals 1951), but sprays are needed to kill young larvae that might not be noticed in routine inspection. A 2-day residual effect is also necessary to assure kill of larvae that hatch from eggs present at time of treatment. The recommended insecticides are effective for this purpose, but they are rather expensive. Experiments with other phosphorus insecticides to develop safe and inexpensive treatments are described in this paper.

TESTS WITH ARTIFICIALLY INFESTED SHEEP.—Insecticides that had shown activity in screening tests against other livestock insects were given preliminary evaluations with small numbers of artificially infested sheep. The wool was clipped from an area on one hip of a healthy sheep and a piece of skin about 1-inch in diameter was removed to expose the muscle. The wound was infested with approximately 200 newly hatched screw-worm larvae. On the following day a similar wound was made and infested on the other hip. When the larvae in one hip were 2 days and in the other 3 days old, the sheep was sprayed at low pressure with enough test material to wet the wound and surrounding area thoroughly.

The sheep were examined at intervals after treatment, with a final examination at 24 hours. With most sprays the insects were visibly affected within 15 minutes and the larvae were killed or driven from the wound within 6 hours. The animals that were still infested after 24 hours were removed from the test. Every treatment effective at this time was challenged by introducing 200 newly hatched larvae on a fresh, raw spot made by scraping debris from a ½-inch area in the wound. The wound was examined on the following day, and if larvae had established, the animal was removed from the test. If the larvae had died, the treatment was challenged each day for 7 days or until it failed.

Nine insecticides were evaluated by this method: Co-

[1] Accepted for publication May 31, 1960. In cooperation with the Animal Disease Eradication Division, Agricultural Research Service.

Table 1.—Comparison of Co-Ral with other insecticides for control of screw-worms in artificially infested sheep. Summer 1959.

Treatment	Number of Wounds Reinfested / Number of Wounds Challenged (After Days Shown)						
	1	2	3	4	5	6	7
Co-Ral, 0.25%	0	0	1	0	0	0	0
	–	–	–	–	–	–	–
	4	4	4	2	2	2	2
	0	0	0	0			
	—	—	—	—	—	—	—
	10	10	10	10			
Bayer 22408							
0.5%	0	0	0	0	1	2	
	–	–	–	–	–	–	—
	4	4	4	4	4	2	
1%	0	0	0	0	0	0	0
	–	–	–	–	–	–	–
	4	4	4	4	4	4	0
Ethion, 0.5%	0	0	1	1			
	–	–	–	–	—	—	—
	4	4	4	2			
General Chemical 3583, 0.1%	0	0	1	0	1		
	–	–	–	–	–	—	—
	4	4	4	2	2		
General Chemical 4072, 0.5%	0	0	2	1			
	–	–	–	–	—	—	—
	4	4	4	2			
Malathion, 0.5%	0	0	3				
	–	–	–	—	—	—	—
	4	4	4				
Ruelene, 1%	0	1	0	1			
	–	–	–	–	—	—	—
	4	4	2	2			
Sevin, 2%	0	1					
	–	–	—	—	—	—	—
	2	2					
Dimethoate, 0.25%	1	2					
	–	–	—	—	—	—	—
	7	5					
.5%	0	1	0	0			
	–	–	–	–	—	—	—
	8	8	7	7			
1%	0	2	0	0			
	–	–	–	–	—	—	—
	9	9	6	6			

Ral, Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate), ethion, General Chemical 3583 (diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate), General Chemical 4072 (2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate), malathion, Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), Sevin® (1-naphthyl methylcarbamate) and dimethoate.

All treatments, except Sevin and dimethoate, were toxic to both ages of larvae present at the time of spraying. Sevin and dimethoate failed to kill all 3-day-old larvae, and some 2-day-old larvae survived in wounds treated with 0.25% dimethoate. Data on the residual effect of the nine insecticides are summarized in table 1.

Table 2.—Field tests on several ranches in Texas comparing the effectiveness of Bayer 22408 and Co-Ral for screw-worm control. Fall 1959.

Treatment	Animals	Number of Screw-Worm Cases After (Days) 3	5	7	12	15
	Ranch 1, Montell					
Bayer 22408, 0.5%	285 ewes	0	0	0	5	1
Co-Ral, 0.25%	266	0	0	0	2	1
	Ranch 2, Fredericksburg					
Bayer 22408, 0.25%	106 wethers	0	0	0	0	0
	Ranch 3, Uvalde					
Bayer 22408, 0.5%	10 goats	0	0	1	2	0
1%	30	0	0	1	3	0
	7 cattle	0	1	1	3	1
	Ranch 4, La Pryor					
Bayer 22408, 0.5%[a]	24 steers	0	0	0	13	3
1%	23	0	0	0	5	0
Co-Ral, 0.5%	23	0	0	0	12	1
	Ranch 5, La Pryor					
Bayer 22408, 0.5%	150 steers	2	0	4	11	9
Co-Ral, 0.25%	100	2	8	0	0	0
EQ-335	19	3	4	5	7	1
	Ranch 6, Batesville					
Bayer 22408, 0.25%	30 steers	0	0	0	0	1
Co-Ral, 0.25%	30	0	1	0	0	0
EQ-335	10	0	0	1	1	0
	Ranch 7, Eagle Pass					
Bayer 22408, 0.25%	39 steers	0	0	3	2	0
Co-Ral	25	0	0	2	1	0
EQ-335	18	0	0	11	7	1

[a] A group of 70 steers had 5 screw-worm cases after 3 days and 19 after 5 days; test discontinued.

Bayer 22408 appeared especially promising, and since it was available in sufficient quantities, it was tested further on larger numbers of artificially infested sheep with similar results. Before field trials were undertaken, cooperating veterinarians[2] determined safe concentrations for use on sheep, goats, and baby calves and found them to be four to eight times those of Co-Ral.

FIELD TESTS WITH SHEEP, GOATS, AND CATTLE.—Livestock on seven ranches in southwestern Texas were treated with Bayer 22408 and Co-Ral and maintained in accordance with usual ranch practices. On some ranches EQ-335 was also used. The animals were already infested with screw-worms or bore fresh wounds susceptible to infestation, such as shear cuts, and castration, branding, and dehorning wounds.

Co-Ral and Bayer 22408 wettable powders were applied with a power sprayer at a pressure of 250 pounds per square inch to wet the penned animals thoroughly. EQ-335 was applied with a brush. If two or more treatment groups were pastured together, the animals were marked with different color paints.

The rancher penned and examined his animals twice a week for at least 15 days after spraying and kept records on the control of existing infestations and duration of protection. If a wound was infested with screw-worms, he treated it with EQ-335 and removed the animal from test. After 15 days most wounds were sufficiently healed that

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Table 3.—Laboratory tests with artificially infested sheep to compare the effectiveness of Bayer 22408 and Co-Ral. Fall 1959.

Treatment	Number of Wounds Reinfested / Number of Wounds Challenged (After Days Shown)						
	1	2	3	4	5	6	7
Bayer 22408, 0.1%	0/10	1/10	0/8	3/8	0/3	0/3	0/3
.25%	0/10	0/10	0/10	2/8	0/6	0/6	0/6
	0/40	0/40	0/40	0/40	0/40	0/40	0/40
	0/20	0/20	1/20	0/18	0/16	0/16	3/10
	0/36	0/30	0/28	0/24	2/16	0/12	1/10
.5%	0/18	1/18	0/16	0/16	0/16	0/16	2/12
	0/24	0/22	1/22	0/18	0/10	0/10	0/10
	0/20	0/20	0/20	0/20	0/20	1/20	1/18
	0/39	0/31	0/31	0/25	2/19	0/17	1/17
1%	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Co-Ral, 0.25%	0/40	0/40	1/38	1/36	1/34	6/22	0/16

Table 4.—Laboratory tests with Bayer 22408 sprays on artificially infested cattle. Fall 1959.

Treatment	Number of Wounds Reinfested / Number of Wounds Challenged (After Days Shown)					
	1	2	3	4	5	6
0.1%	3/19	2/16	0/12	5/12	0/4	1/4
.25%	1/29	1/23	10/17	—	—	—
	7/23	0/6	0/6	1/6	—	—
.5%	2/30	0/24	7/24	—	—	—

they were no longer susceptible to infestation. As soon as possible after the observation period, an entomologist conferred with the rancher regarding his records.

The results of these tests are presented in table 2. All the treatments were reported by the ranchers to be effective against infestations present at time of spraying. Also, on five ranches no animals were reinfested at the 3-day examination. However, on Ranches 4 and 5, which were subjected to heavy reinfestation, animals in four groups of steers had some infested wounds after 3 days. Since the rancher did not estimate the age of screw-worm larvae, it is not known how soon after spraying those wounds became infested. Except on these ranches the new treatments performed about as well as Co-Ral and both insecticides were superior to EQ-335.

LABORATORY COMPARISON OF BAYER 22408 AND CO-RAL.—Because of the failure of Bayer 22408 on cattle in 3 days on two ranches, laboratory studies were made on its residual effect on cattle and sheep.

A total of 122 sheep were wounded and infested according to procedures described above, and were sprayed with Bayer 22408 at concentrations ranging from 0.1 to 1%. For comparison, 20 sheep similarly infested were sprayed with 0.25% Co-Ral. When the animals were examined after 24 hours, all the treatments had killed all the 2-day-old larvae. Some 3-day-old larvae survived in 2 of 50 wounds sprayed with 0.25% Bayer 22408 and in 3 of 52 wounds sprayed with 0.5% Bayer 22408. Those five animals were removed from test, and the treatments on the remaining animals were challenged as described above. The results of the residual effects of the treatments are shown in table 3.

Twenty yearling cattle were each wounded six times by removing discs of skin. Three wounds were in exposed locations, such as on the hip, side, or shoulder, and the others were hidden, such as on the belly or inside the flank. At the time of spraying each animal bore an open and a hidden wound infested with 1-day-old larvae, two similar wounds infested with 2-day-old, and two with 3-day-old larvae.

The cattle were divided into four groups of five each and sprayed with 22408 at concentrations of 0.1, 0.25, and 0.5%. The insecticide was applied with a power sprayer and 1½ gallons was used to wet each animal thoroughly. Earlier field tests had shown that thorough spraying of cattle with 0.25% Co-Ral killed all three ages of larvae and gave at least 2 days' residual protection from reinfestation (H. M. Brundrett, unpublished).

The results of tests with Bayer 22408 on cattle are presented in table 4. Only the 0.5% spray killed all three ages of larvae. However, even that treatment did not prevent establishment of larvae when it was challenged on the following day. Therefore, this insecticide could not be recommended as satisfactory for use on cattle at 0.5% concentration.

The conflicting results on cattle and sheep are difficult to explain. The failure of residues of Bayer 22408 to per-

sist on cattle hair might be owing to physical differences in deposits from wettable powder sprays made with the 50% or the 25% Co-Ral. Further research is needed in precise laboratory studies on the relative efficiency of the two insecticides and in field tests of different spray formulations on cattle.

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Control of *Oligonychus pratensis* Attacking Winter Wheat in Western Kansas[1]

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ABSTRACT

A mite, *Oligonychus pratensis* (Banks), has been causing concern among wheat growers in western Kansas during recent years. Heavily infested plants have been killed by a combination of mite feeding and insufficient soil moisture.

In 1958 demeton, Trithion,® parathion, Kelthane,® Guthion,® Tedion,® and malathion reduced mite populations significantly 3 days after application but none provided satisfactory control and none was effective 14 days after treatment. In 1959, all materials reduced mite populations 4 days after application but only Trithion, Kelthane, and parathion provided high initial mortality. Demeton gave fair control but Phostex® and Delnav® were ineffective. Trithion and Kelthane were superior to all other materials 14 days after treatment.

Parathion applied at 1.0 lb. per acre by airplane significantly reduced mite populations and gave a substantial increase in seed yield.

Definitions of chemicals bearing proprietary names above are as follows:

Delnav (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate 68%–70% and related materials)).

Guthion (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate).

Kelthane (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol).

Phostex (a mixture of bis(dialkyloxyphosphinothioyl) disulfides).

Tedion (2,4,4′,5-tetrachlorodiphenyl sulfone).

Trithion (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate).

A mite, *Oligonychus pratensis* (Banks),[3] has caused increasing concern to wheat growers in western Kansas during recent years. Banks (1912) originally described the mite as *Tetranychus pratensis* from material collected on timothy, *Phleum pratense*, at Pullman, Washington. Since then Ewing (1913) and McGregor (1920, 1950) have published revised descriptions.

Very little has been published on injury or control, particularly on winter wheat. Griffith & Wene (1953) reported it injuring sorghum near San Manuel, Texas. They reported that sulfur, applied at 35 pounds per acre, showed apparent toxicity to the mite. Harvey (1954) reported it was the most injurious pest of the 1952–53 wheat crop in eastern New Mexico. He reported that demeton (Systox) and parathion consistently gave the best control among the materials field tested. Walter (1956) reported it distributed throughout the lower Rio Grande Valley and that fields of corn were seriously injured. A heavy dusting of sulfur did not provide effective control.

In October, 1957, winter wheat attacked by numerous colonies of mites near Marienthal in Wichita County, Kansas showed characteristic white "stippled" leaves and webbing in the crowns. Many leaves had developed chlorosis and heavily infested plants were killed by mite feeding. Lack of soil moisture undoubtedly aggravated the damage.

Adjacent grain sorghum was the source of infestation. Mites were most common along the margin of the wheat field adjacent to the sorghum and proportionately smaller numbers were found farther into the seeded wheat.

Reports received during 1958 and 1959 indicated that this mite was rather generally distributed in western Kansas, causing injury to seeded wheat. Figure 1 shows damage to wheat caused by this mite. In many cases the infestation in seeded wheat originated from volunteer wheat rather than from grain sorghum. A combination of drouth and mite feeding had killed the thick volunteer wheat and the mites had migrated to seeded wheat for more succulent plants to feed on.

Results of preliminary investigations to determine the effectiveness of various acaricides are reported in this paper.

[1] Contribution No. 767, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan; No. 38, Garden City Branch Experiment Station, Garden City, Kansas. Accepted for publication May 31, 1960.

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[3] Determined by E. W. Baker, Insect Identification and Parasite Introduction Laboratories, U. S. Department of Agriculture. Often in literature *Paratetranychus pratensis*, called timothy mite or date mite. Date mite is the common name adopted by the Entomological Society of America.

FIG. 1.—Wheat field showing portion destroyed by fall infestation of *Oligonychus pratensis*. Picture taken in spring near Montezuma, Kansas.

MATERIALS AND METHODS.—*Fall Application, 1958.*—This investigation was conducted in a field of Wichita wheat near Montezuma, Kansas. The test involved seven acaricides and one untreated check replicated three times in a randomized block design. Plot size was 11 rows wide and 25 feet long. All acaricides were applied October 30 with a 2-gallon, compressed-air hand sprayer equipped with an 18-inch boom. The rate of output was 17 gallons per acre at a pressure setting of 20 p.s.i. Atlox 1045-A was added to all sprays as a wetter and spreader.

The average maximum and minimum temperatures were 62.9° and 46.6° F., respectively. No precipitation occurred during the test period. Wind was estimated at approximately 3 to 5 m.p.h.

Mite counts were made November 3 and 13. Population sampling consisted of a washing and extraction technique described by Henderson (1957). Counting the mites directly on the wheat plants was not practical. Percentage control was based on differences in numbers of live mites between treated and untreated plots.

Spring Application, 1959.—Plots were located in new locations of the same field to avoid contamination by previous applications. Application of materials was made April 24. Average maximum and minimum temperatures were 77.6° and 46.5° F., respectively. Precipitation measured 0.40 inch. Wind velocity was estimated at 5 m.p.h.

Aerial Spraying.—The effectiveness of parathion applied by aircraft was also investigated. Each treatment included approximately 9 acres and was not replicated. Parathion was applied at 0.5, 0.75, and 1.0 lb. actual per acre in diesel oil at 2 gallons per acre on headed wheat, May 26, 1959. Wind velocity was estimated at 5 to 10 m.p.h.

Wheat samples were harvested for yield data 1 month later (June 26, 1959) by hand-cutting individual plots. Eight ½-rod row samples were cut from each plot, making a combined sample of 66 feet of row per treatment. Each ½-rod row sample was chosen at random. The grain was threshed with a custom-built, beating-type thresher, after which the seed was cleaned and weighed. Yields, in grams per plot, were determined and then converted to bushels per acre.

RESULTS AND DISCUSSION.—*Fall Application, 1958 Experiment.*—Data given in table 1 show that all treatments were significantly better than the check, but none was particularly effective. Demeton gave the best results 3 days after treatment. Trithion® (*S*-(*p*-chlorophenylthio)-methyl *O,O*-diethyl phosphorodithioate) ranked second, followed by parathion.

None provided satisfactory control 14 days after application. Demeton and Trithion gave the best results with Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone) ranking third. Malathion gave the poorest control. Cool weather and the movement of mites to protected areas in the crown of the plants may have contributed to the poor results obtained.

Spring Application, 1959 Experiment.—Statistical analyses indicated that all treatments were significantly better than the check (table 1). Trithion was the most effective 4 days after application. At a dosage somewhat heavier than used in 1958, parathion and Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol) were a significant improvement over the lower rate. Unlike the 1958 results, demeton gave only fair control, perhaps because the supply used had aged.

Kelthane gave control equal to Trithion 14 days after application. Mite infestations began to increase steadily in the parathion-treated plots. It and demeton gave only fair control.

Aerial Spraying.—Control was evident following application of parathion at 0.5, 0.75, and 1.0 lb. per acre (table 2). However, only the 1.0-lb. dosage provided effective

Table 1.—Control of *Oligonychus pratensis* on winter wheat, Montezuma, Kansas.

Treatment[a]	Active Toxicant (lb. per acre)	Days After Treatment: 3 — No. of Mites[b]	3 — Per Cent Control	14 — No. of Mites[b]	14 — Per Cent Control
		1958 Test			
Demeton	0.375	96	77	120	68
Trithion	0.5	115	72	138	63
Parathion	0.5	128	69	178	47
Kelthane	0.5	176	57	167	55
Guthion®[c]	0.375	177	57	178	52
Tedion	0.5	184	55	156	58
Malathion	1.0	247	40	224	40
Check	—	412	—	374	—
L.S.D. at 5%		40		60	
at 1%		55		83	
		1959 Test[d]			
Trithion	0.5	17	90	14	94
Parathion	0.75	27	85	47	78
Kelthane	0.75	30	83	14	94
Demeton	0.375	40	77	50	77
Phostex®[e]	0.75	66	63	109	50
Delnav®[f]	1.0	94	47	110	49
Check	—	178	—	218	—
L.S.D. at 5%		38		38	
at 1%		57		53	

[a] All emulsifiable concentrates except Tedion, which was a wettable powder, 50%.
[b] One pint aliquot sample (average of 3 replicates).
[c] (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate).
[d] First posttreatment count 4 instead of 3 days after application.
[e] (A mixture of bis(dialkyloxyphosphinothioyl) disulfides).
[f] (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate 68%–70% and related materials)).

control 3 days after application. Fourteen days after application all treatments showed a slight increase in control. Parathion at 1.0 lb. per acre remained the only effective treatment. Mite populations in the untreated as well as the treated plots dropped when a 14-day count was made so no further counts could be evaluated.

Data on seed yields are summarized in table 2. Each

Table 2.—Effectiveness of parathion aerial applications to control *Oligonychus pratensis* on winter wheat, Colby, Kansas, 1959.

Treatment	Active Toxicant (lb. per Acre)	Days After Treatment: 3 — No. of Mites[a]	3 — Per Cent Control	14 — No. of Mites[a]	14 — Per Cent Control	Yield (bu./a.)
Parathion	0.5	62.3	40	21.0	46	21.8
	0.75	40.0	61	11.8	70	22.9
	1.0	18.0	83	6.3	84	27.1
Check	—	103.5	—	38.8	—	18.9
L.S.D. at 5%		20.0		9.6		
at 1%		28.7		13.8		

[a] One pint aliquot sample (average of 4 samples).

treatment increased yield over the untreated check. However, only the parathion treatment at 1.0 lb. per acre gave a substantial gain.

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Studies of the Biology of the Tarnished Plant Bug, *Lygus lineolaris*[1]

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Abstract

Because of the importance of the tarnished plant bug, *Lygus lineolaris* (P. de B.), as a pest of the seed of birdsfoot trefoil, *Lotus corniculatus* L., studies were initiated on certain aspects of the bug's biology.

The seasonal history was observed in the field from weekly bug collections and by dissecting the females to study egg development. All of the information suggests that there are at least two and probably three generations each year of the tarnished plant bug associated with birdsfoot trefoil in New York.

An experiment designed to determine the height of flight of the tarnished plant bug indicated that about 90% of these insects fly within 6 feet of the ground and few, if any, fly higher than 18 feet.

Preliminary investigations of the sex ratio revealed that, averaged over the growing season, the males of the tarnished plant bug outnumbered the females. However, females significantly outnumbered males in groups of tarnished plant bugs collected from tanglefoot traps in the spring prior to the appearance of the first new adults of the season.

[1] Accepted for publication June 2, 1960.
[2] The authors gratefully acknowledge the assistance of S. W. Jacklin, C. S. Koehler, H. H. Shorey, and H. Y. Forsythe, Jr. in certain phases of the field work.

The tarnished plant bug, *Lygus lineolaris* (P. de B.), is an important pest of birdsfoot trefoil, *Lotus corniculatus* L., grown for seed (Neunzig *et al.* 1958, MacCollom 1958, Ridgway & Gyrisco 1959). Because of its importance as a pest of birdsfoot trefoil, studies were initiated on certain aspects of the biology of this plant bug, particularly as it is associated with birdsfoot trefoil. These experiments included investigations of the seasonal history of the tarnished plant bug, determination of the number of eggs in the ovaries of the females throughout the growing season, studies of height of flight, and of the sex ratio.

METHODS AND MATERIALS.—In the investigations of the seasonal history, 100 sweeps with a 15-inch diameter insect net were taken in a total of six widely spread, birdsfoot trefoil fields, at approximately weekly intervals, in 1958 and 1959. The primary objectives of these collections were to determine the population levels throughout the growing season and to get information concerning the number of generations each year. Additional observations were also made which will be used to help outline the seasonal history. For the determination of the numbers of eggs possessed by females throughout the growing season, insect collections were also made with the net. The females were dissected under a stereoscopic microscope and the numbers of eggs present were determined.

In the height of flight studies, traps were constructed from 2- by 4-inch wood stock, to which 2- by 6-foot panels covered with 14-mesh-screen wire were nailed. The flight traps were erected perpendicular to the ground and were supported by four guy wires. Both sides of the structures were covered with tanglefoot; thus, a surface approximately 2 feet wide and 18 feet high was exposed to serve as a trap for flying insects. The structures were erected on July 24, 1959 in an alfalfa field and located so that two of the traps were facing north and south and the other two were facing east and west. On July 31 and August 7 the number of tarnished plant bugs present in every 3 foot increment were counted and removed.

In determining the sex ratio two methods of collection were used: (1) collection with the net and (2) collection from the tanglefoot traps.

RESULTS.—*Seasonal History.*—Counts of nymphs of the tarnished plant bug were taken at approximately weekly intervals during the growing seasons of 1958 and 1959

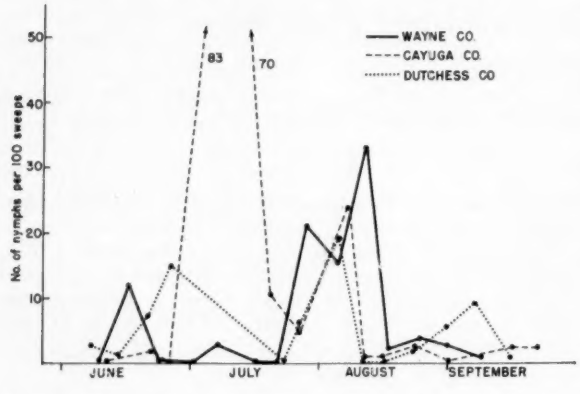

FIG. 1.—The number of nymphs of the tarnished plant bug collected at approximately weekly intervals from birdsfoot trefoil in three locations in New York. 1958.

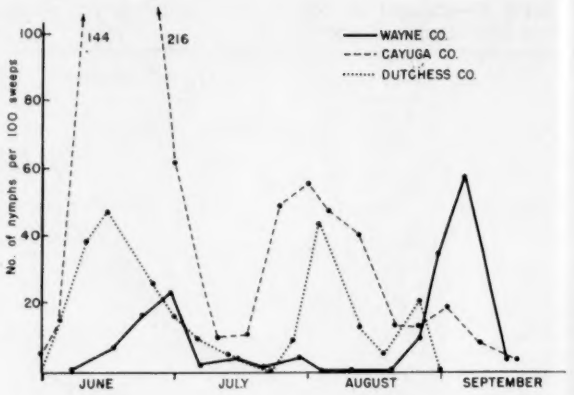

FIG. 2.—The number of nymphs collected at approximately weekly intervals from birdsfoot trefoil in three locations in New York. 1959.

and are presented graphically in figures 1 and 2. Only the counts of nymphs are presented because it is believed that these non-migratory stages are more reliable in demonstrating peaks in population from a particular generation. From the information presented in figures 1 and 2 and other unpublished observations (Ridgway & Gyrisco 1958) it is now possible to outline the seasonal history of the tarnished plant bug in New York.

The overwintering adults begin laying eggs around the first of May. Because of their extremely small size first instars are difficult to find, but the eggs are believed to begin hatching shortly after the middle of May. However, the first generation of nymphs do not reach noticeable numbers until after mid-June. The first new adults of the season appear about this same time. The second generation of nymphs reach peak numbers about the first of August. After the second generation it is difficult to describe accurately the events as they occur in birdsfoot trefoil. In some locations there appears to be a third peak of nymphs after the first of September. In view of the fact that nymphs may be found on weed hosts late into the fall and that a high percentage of unfertilized females may be found in birdsfoot trefoil at three times during the season (table 1) it is believed that a third generation probably exists. In Ontario, Canada, Guppy (1958) reported two generations per year of the tarnished plant bug on forage crops. Salt (1943) also reported two generations per year for *Lygus hesperus* Knt. and *L. elisus* Van D. in Alberta, Canada.

It should be noted that mowing practices of birdsfoot trefoil remove eggs as well as food from the field and may affect and obscure the number of generations of the tarnished plant bug. However, it appears that while mowing practices may skew the population peaks in individual fields, they are not believed to alter drastically the biology of the bug.

Number of Eggs per Female.—The numbers of eggs which were found in dissected females are recorded in table 1. In view of the 8- to 10-day preoviposition period, the absence of females not carrying eggs during the early part of the season supports the observation that the tarnished plant bug overwinters as an adult. The appearance of females not carrying eggs on June 3 and June 10

Table 1.—Number of eggs found by dissection of tarnished plant bug females. Aurora, New York. 1959.

Date of Collection	No. of Females Dissected	No. of Eggs		Per Cent of Females Without Eggs	Mean Number of Eggs per Female Among Those With Eggs
		Range	Mean per Female		
4-25	10	10–26	15.9	0	15.9
4-30	7	10–19	14.1	0	14.1
5- 9	6	11–27	16.7	0	16.7
5-14	8	6–24	11.4	0	11.4
5-20	5	3– 8	6.2	0	6.2
5-28	7	5–16	12.3	0	12.3
6- 3	17	0–36	8.1	11	9.1
6-10	15	0–14	4.3	27	6.2
6-20	15	0–16	1.1	93	16.0
7- 2	20	0–15	5.6	40	9.3
7- 9	10	0–19	6.2	60	15.5
7-15	10	0–19	9.3	40	15.5
7-23	20	0–19	8.6	30	11.4
7-29	20	0–19	10.6	25	14.1
8- 5	20	0–19	9.0	25	12.0
8-12	20	0–18	7.1	45	12.8
8-19	20	0–13	2.8	70	9.3
8-26	20	0–16	6.0	50	12.0
9- 2	20	0–19	7.3	40	12.2
9- 9	20	0–16	2.0	25	8.0
9-17	10	0	—	100	—

indicates that these individuals were either overwintering females which were no longer gravid or else new females unfertilized. Based on coloration, the latter was definitely true of some specimens collected on June 10.

The presence of a high percentage of females not carrying eggs and new bright coloration in late June, mid-August, and mid-September indicates broods of new adults. The absence of eggs in females collected on September 17 does not mean that no eggs were laid after that date, since some oviposition was observed to continue into late fall.

The mean number of eggs found in females throughout the growing season was 7.84, and the mean number of eggs among only females which possessed eggs was 10.7.

Table 2.—Total number of tarnished plant bugs removed from tanglefoot traps at various heights. Oswego, New York. 1959.

Height in Feet	Trap Number				
	1	2	3	4	Total
0– 3	125	88	53	58	224
3– 6	30	26	8	12	76
6– 9	4	6	4	2	16
9–12	2	2	0	0	4
12–15	0	1	1	0	2
15–18	0	0	0	1	1

Table 3.—Total number of males and females of the tarnished plant bug from weekly insect collections. Aurora, New York. 1959.

Category	Males	Females	Chi Square
April to September (from net sweeps)	661	585	4.64[a]
May to early June from tanglefoot traps (overwintering population)	222	348	27.86[a]

[a] Significant at the 5% level.

Height of Flight.—The numbers of tarnished plant bugs taken at the different heights of flight are presented in table 2. Few, if any, flew more than 18 feet from the ground. By far the largest number (more than 90%) flew no higher than 6 feet from the ground. Crosby & Leonard (1914) reported that very few tarnished plant bugs were observed to fly over a cloth-wire fence about 6 feet tall which was erected to exclude them from a nursery.

Sex Ratio.—The total numbers of males and females of the tarnished plant bug collected are recorded in table 3. The males significantly outnumbered the females in collections made with a sweep net throughout the season. Bug collections were made also from the tanglefoot traps and sexes determined from the first of May until the time that the first new adults of the season appeared. The Chi-square value computed indicated in this instance that the females significantly outnumbered the males. Therefore, either the females in the overwintering population outnumbered the males or more females fly in spring than males. The numbers of tarnished plant bugs involved in these studies of the sex ratio were small and therefore, these results may not be conclusive.

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Toxicity to House Fly Larvae of Droppings from Chicks Given Dipterex-Treated Water[1]

MARTIN SHERMAN and ERNEST ROSS[2]

ABSTRACT

The effect of continuous administration via the water supply to chicks of Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) on the toxicity of the manure to house fly (*Musca domestica* L.) larvae was investigated. The hydrogen ion concentration of the water used in the Dipterex solutions influenced the toxicity of the manure. Water solutions at pH 8.0 resulted in a rapid decrease in toxicity to larvae. However, Dipterex at 30 p.p.m. in distilled or acidified water at pH levels below 7.0 caused high levels of larval mortality in the droppings. At this concentration, no adverse effect on weight-gain, water consumption, or feed conversion was noted in the chicks. It appears that the chick is able to metabolize Dipterex and excrete a highly larvicidal product but when conversion to DDVP occurs under alkaline conditions the metabolic pathway is different and results in products having low larvicidal activity.

The incorporation of insecticides in poultry feed has been shown to be an effective means of controlling house fly (*Musca domestica* L.) larvae in manure. Sherman & Ross (1960) and Ross & Sherman (1960) described the results of several experiments in which a number of insecticides were orally administered in the feed. High larval mortality was obtained at relatively low levels. The use of insecticides distributed in the water supply of poultry for larval control has definite advantages over their use as feed additives, *i.e.*, ease in preparation, more accurate admixture, and more flexibility in administration. Although water solubility is not essential in a candidate insecticide since emulsifiable formulations could be used, it would be a beneficial property since no adjuvants would be required. Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) which was found in the forementioned studies to be highly larvicidal when used as a feed additive exhibits the property of high water solubility. This study was undertaken to investigate the effect of continuous administration of Dipterex in the water supply of chicks on the toxicity of the manure to house fly larvae.

METHODS AND RESULTS.—The chicks used throughout the study were the University of Hawaii strain of New Hampshires. All chicks received, from 1 day of age, a starter ration of the following composition: ground corn, 60%; soybean oilmeal, 36%; defluorinated phosphate, 3%; iodized NaCl, 0.5%; and vitamins and trace minerals, 0.5%. Feed and water were supplied *ad libitum*.

The Dipterex used in all experiments was a technical grade material containing 100% active ingredients. Stock solutions were prepared in distilled water and then diluted to the required concentrations with distilled water, tapwater, and acidified tapwater as described below. Fresh solutions of all treatments were prepared weekly throughout the course of each experiment. The treated water was stored in 5-gallon glass bottles atop the chick batteries and conducted to the watering troughs by means of rubber tubing. The watering troughs were filled by siphoning from the bottles, when necessary. No other water was available to the chicks in these experiments.

Droppings were collected from the dropping pans periodically and assayed for toxicity to house fly larvae. The droppings from each cage were collected separately in half-pint waxed cartons and brought into the laboratory. Triplicate fecal samples of 15 to 20 grams each, were taken from each carton, placed in a Stender dish, and approximately 2 ml. of distilled water added. Ten newly hatched house fly larvae were placed in each fecal sample. The dish was then covered with a fine muslin cloth to prevent the escape of the larvae and stored for 3 days at a temperature of 25° C. Two days after the introduction of the larvae, an additional 2 ml. of water were added to each dish to replace moisture lost by evaporation. Three days after the introduction of the larvae into the fecal sample, the number of survivors was determined. The controls in each test consisted of larvae-inoculated droppings from the basal groups on insecticide-free drinking water. All larval mortality was corrected for natural mortality by the use of Abbott's formula (Abbott 1925).

In addition to the periodic assays of the toxic properties of the feces, weekly observations were made in all experiments on chick body weight, water consumption, and feed consumption. The gains in weight of the chicks were expressed as the ratio of the final body weight divided by the initial body weight for any given time-period. In experiments 2 and 3, the weight-gain data were analyzed by means of the one-tailed "t" test (Cochran & Cox 1950). Feed consumption was related to body weight in terms of grams of feed per gram of gain in body weight. This ratio expresses the efficiency of feed utilization or feed conversion.

Experiment 1.—In this experiment an attempt was made over a 3-week period to determine the range of concentrations of Dipterex which would be innocuous to the chick and yet result in manure which would be toxic to house fly larvae. For this preliminary study, twenty-eight 3-week-old chicks of mixed sex were placed in a modified individual chick battery without heat. Two chicks were distributed at random into each pen and two pens were assigned to each treatment. Distilled water solutions of Dipterex were prepared in the following concentrations: 33, 333, 3, 333, 1, 667, 333, 167, 33, and 0 p.p.m.

The results of this experiment are summarized in table 1. The droppings from the chicks receiving all concentrations of Dipterex-treated water caused 100% mortality in the house fly larvae. Treated water containing 1,667 p.p.m. or higher resulted in 100% mortality to the chicks,

[1] Presented at the forty-fourth annual meeting of the Pacific Branch, Entomological Society of America held at Spokane, Washington, June 28–30, 1960. Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 488. Accepted for publication June 3, 1960.

[2] Entomologist and Associate Poultry Scientist, respectively. The writers are indebted to Mr. George Komatsu for his assistance.

Table 1.—Effect of Dipterex-distilled water solutions on New Hampshire chicks and the toxicity of resulting droppings to house fly larvae.

Concentration (p.p.m.)	No. Larvae Tested	Mortality (%)		Mean Water Consumption (ml./chick/day)	Mean Weight-Gain (gms.)[a]	
		Larval	Chick		♂	♀
0	80	5	0	95	444	415
33	80	100	0	85	396	369
167	80	100	0	80	286	255
333	70	100	0	63	467	265
1,667	20	100	100[b]	12	—[c]	—[c]
3,333	20	100	100[b]	10	—[c]	—[c]
33,333	20	100	100[b]	21	—[c]	—[c]

[a] Mean weight-gain per chick during the 3-week period of the experiment.
[b] All birds died within 8 days of start of experiment.
[c] All birds lost weight prior to death.

all deaths occurring within 8 days of the start of the experiment. Affected chicks exhibited the same symptoms caused by single oral dosages of Dipterex described earlier by Sherman & Ross (1959).

There appeared to be an inverse relation between water consumption and Dipterex concentration. Although few birds were used in this experiment, the weight-gain data also indicate an inverse relationship between gain and Dipterex concentration.

Experiment 2.—This experiment was designed to determine, more accurately, that concentration of Dipterex which would result in the most effective larvicidal activity of the manure. One hundred and seventy 8-day-old chicks were distributed at random in an electrically-heated and thermostatically-controlled, raised-wire-floor Petersime battery. One series consisting of males was divided into five treatment-groups of 17 chicks each, and a second series consisting of females was divided in the same manner. Within each series, the drinking water was treated so that the chicks received the following concentrations of Dipterex: 30, 20, 10, 3, and 0 p.p.m. In this experiment, the solutions were made up in tapwater instead of in distilled water. These treatments were continued for 2 weeks.

It was found that at 30 p.p.m., Dipterex in tapwater caused approximately 20% mortality in larvae placed in the droppings. This was similar to the larval mortality occurring in the droppings from the chicks receiving the untreated water. Therefore, the concentrations were changed to 30, 90, 62, 43, and 0 p.p.m., respectively, and continued for an additional 3 weeks. One of the solutions originally containing 30 p.p.m. Dipterex in tapwater was prepared in distilled water to determine whether the solvent had any effect on larval mortality. All other concentrations were prepared in tapwater. Daily collections of droppings were made and these were assayed for larval toxicity. Figure 1 correlates Dipterex concentration, larval mortality, solvent, and age of solution. Natural larval mortality occurring in the untreated control droppings ranged between 8% and 18%. The distilled water solution containing Dipterex at 30 p.p.m. maintained a high toxicity to the larvae over a 7-day period. The manure from chicks on this same concentration in tapwater quickly lost its toxic properties. There was a definite correlation between Dipterex concentration in tapwater, age of solution, and loss in toxicity of the droppings. A decrease in activity occurred with an increase in time. This decrease was slower at the higher

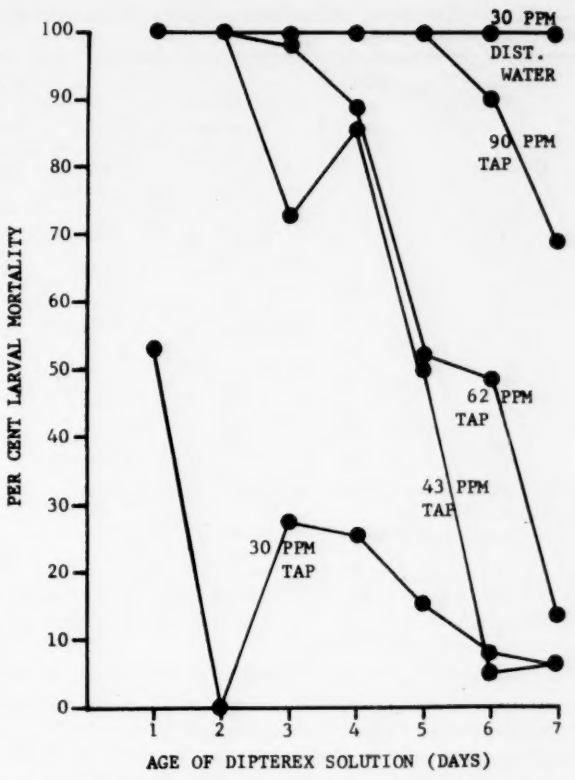

FIG. 1.—Effect of age of tapwater solutions of Dipterex consumed by chicks on mortality of house fly larvae in the droppings. Larval mortality corrected for natural mortality.

concentrations of Dipterex. No chick mortality occurred during the 5-week period of the experiment.

Table 2 summarizes the effect of tapwater solutions of Dipterex on weight-gain ratio, water consumption, and feed conversion in the chicks. At concentrations as high as 30 p.p.m., there was no adverse effect on weight-gain or efficiency of feed utilization in either male or female chicks. The water consumption followed the same general trend as noted in experiment 1. In both sexes, the water consumption decreased with increasing concentration of Dipterex. At concentrations above 30 p.p.m., there was a highly significant reduction in weight-gain in both sexes. While no effect was noted on feed conversion in the males, there appeared to be a decrease in the efficiency of feed utilization in the females with an increase in Dipterex concentration. Water consumption was generally depressed by an increase in concentration.

Experiment 3.—Since comparable dosages of Dipterex in tapwater resulted in lower larval mortality than in distilled water, a third experiment was conducted to determine the effect on larval mortality of acidifying the tapwater. Fifty 3-week-old male chicks were divided within a Petersime battery into five equal groups which received 30, 20, 10, 3, and 0 p.p.m. of Dipterex in distilled water. Another 50 chicks received the same concentrations of Dipterex in tapwater to which 1 N HCl was added in sufficient quantity to adjust the pH below 7.0. After 2 weeks, the above concentrations of Dipterex were changed to 30, 90, 62, 43, and 0 p.p.m., respectively. The solvents, however, remained the same. After 1 week

Table 2.—The effect of tapwater solutions of Dipterex on weight-gain ratio, water consumption, and feed conversion of New Hampshire chicks.

Pen No.	Period I (chicks 8–22 days of age, 3/31–4/14) Concentration (p.p.m.)	Water Consumption (ml./bird/day)	Weight Gain Ratio	Feed Conversion (gms. feed/gm. gain)	Period II (chicks 22–43 days of age, 4/14–5/5) Concentration (p.p.m.)	Water Consumption (ml./bird/day)	Weight Gain Ratio	Feed Conversion (gm. feed/gm. gain)
				Males				
1	0	52	3.10	1.78	0	95	3.06	2.13
2	3	49	3.72	1.70	42	81	2.80[b]	2.12
3	10	50	3.19	1.81	63	79	2.69[b]	2.16
4	20	45	3.42	1.77	90	69	2.79[b]	2.12
5	30	42	3.33	1.77	30[a]	79	3.15	2.10
				Females				
6	0	50	3.16	1.82	0	116	2.71	2.16
7	3	49	2.97	1.84	42	89	2.34[b]	2.32
8	10	49	3.17	1.75	63	87	2.44[b]	2.28
9	20	44	3.05	1.81	90	89	2.37[b]	2.37
10	30	39	3.00	1.81	30	110	2.73	2.22

[a] Distilled water.
[b] Significantly lower than corresponding material, P = 0.01.

on these concentrations, all treated water was removed and the chicks were given untreated tapwater and observed for an additional week.

The hydrogen ion concentration of the water drawn from the tap was 8.0, while the pH of the distilled and acidified water solutions were 5.7 and 6.6, respectively. Measurements of the fluctuation in pH of the solutions in the bottles and the solutions in the watering troughs were taken over a 1-week period and are presented in figure 2. Despite the differences in the hydrogen ion concentrations of the bottled distilled and acidified tapwater solutions, the daily fluctuations in the pH of the solutions in the trough were remarkably similar. The initial rise in the pH of the solutions in the troughs was likely due to the buffering action of the high protein feed transferred to the troughs by the chicks on their beaks. Since the troughs were not cleaned during the week, the increasing fermentation of the accumulated feed apparently caused a subsequent decrease in the pH of the solutions.

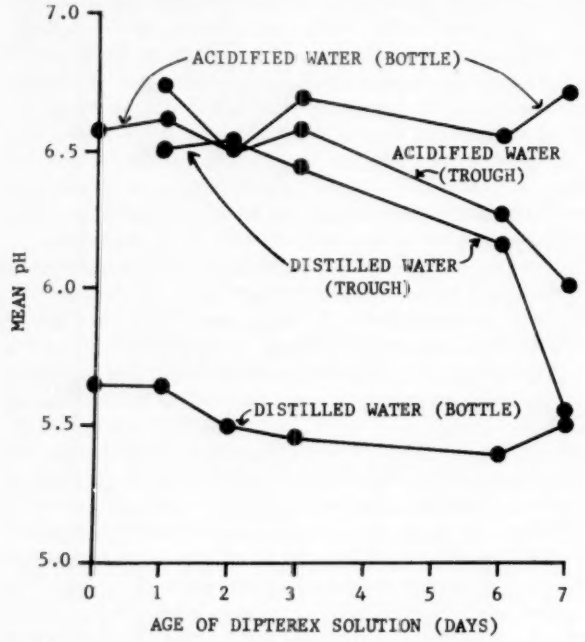

FIG. 2.—Variation in pH of acidified tapwater and distilled-water solutions of Dipterex in storage bottles and watering troughs.

Observations on larval mortality showed no loss in toxicity that could be attributable to the age of the solutions. Table 3 summarizes the toxicity of distilled and acidified water solutions of Dipterex to house fly larvae. There was no apparent difference in toxicity to the house fly maggots between droppings from chicks given distilled water solutions of Dipterex or those given acidified water solutions.

Table 4 summarizes the effect of distilled and acidified water solutions of Dipterex on weight-gain, water consumption, and feed consumption of chicks over a 4-week period. Concentrations up to 30 p.p.m. showed no adverse effect on weight-gain, water consumption, or feed conver-

Table 3.—Effect of distilled and acidified tapwater Dipterex solutions on the toxicity of chick droppings to house fly larvae.

Concentration (p.p.m.)	Dates Tested (1959)	No. Larvae Tested	Corrected Mean Larval Mortality (%)
	Distilled water		
3	4/28–5/12	300	0
10	4/28–5/12	300	28.7
20	4/28–5/12	300	68.0
30	4/28–5/19	510	91.1
43	5/12–5/19	210	100
62	5/12–5/19	210	100
90	5/12–5/19	210	100
	Acidified tapwater		
3	4/28–5/12	300	9.4
10	4/28–5/12	300	29.2
20	4/28–5/12	300	27.5
30	4/28–5/19	510	82.1
43	5/12–5/19	210	100
62	5/12–5/19	210	100
90	5/12–5/19	210	100

Table 4.—The effect of distilled and acidified tapwater solutions of Dipterex on weight-gain ratio, water consumption, and feed conversion of male New Hampshire chicks.

	Period I (chicks 3–5 weeks of age, 4/28–5/12)				Period II (chicks 5–6 weeks of age, 5/12–5/19)				Period III (chicks 6–7 weeks of age, 5/19–5/26)	
Pen No.	Concentration (p.p.m.)	Water Consumption (ml./bird/day)	Weight-Gain Ratio	Feed Conversion (gm. feed/gm. gain)	Concentration (p.p.m.)	Water Consumption (ml./bird/day)	Weight-Gain Ratio	Feed Conversion (gm. feed/gm. gain)	Concentration (p.p.m.)	Weight-Gain Ratio
				Distilled water						*Tapwater*
1	0	107	2.23	1.94	0	141	1.32	2.26	0	1.09
2	3	102	2.24	1.95	43	129	1.31	2.34	0	1.15[b]
3	10	103	2.17	2.02	62	129	1.30	2.38	0	1.19[b]
4	20	96	2.25	2.01	90	128	1.28[b]	2.54	0	1.18[b]
5	30	99	2.22	1.95	30	135	1.31	2.60	0	1.21[b]
				Acidified tapwater						*Tapwater*
6	0	96	2.18	2.03	0	123	1.30	2.32	0	1.10
7	3	104	2.28	1.89	43	141	1.30	2.36	0	1.16[a]
8	10	98	2.19	2.01	62	126	1.26[b]	2.74	0	1.22[b]
9	20	95	2.19	1.98	90	95	1.18[b]	3.73	0	1.27[b]
10	30	100	2.13	2.02	30	148	1.30	2.55	0	1.18[b]

[a] Significantly different from control group, P = 0.05.
[b] Significantly different from control group, P = 0.01.

sion. When the concentrations were changed in the second period, chick growth was significantly depressed at the higher levels. This observation is consistent with the results obtained in experiments 1 and 2. Water consumption and efficiency of feed utilization were also depressed at the higher levels.

When untreated tapwater was substituted in the third period, a highly significant increase in growth occurred in all groups which had previously received the Dipterex-treated water. These data suggest that Dipterex solutions exert a growth depressing effect on chicks which can be reversed by replacing the solutions with untreated water. This could be caused by a physiological effect on the chick or to the unpalatability of the Dipterex solution.

The optimum concentration of Dipterex in distilled or acidified water solution for maximum larval toxicity and minimal toxicity to the chick lies between 30 and 43 p.p.m. These concentrations of Dipterex solutions compared in effectiveness with Dipterex in feed at concentrations of 89 and 132 p.p.m. (Sherman & Ross 1960). A 6-week-old chick normally consumes approximately twice as much water as feed. However, the intake of treated-water is influenced by the concentration of Dipterex (tables 2 and 4). Therefore, a more accurate comparison of the two methods of administration, *i.e.*, via feed or water, would be on the basis of daily intake of Dipterex per bird. Such a comparison showed that the daily intake of Dipterex by either route was essentially the same. At 5 to 6 weeks of age the chicks receiving Dipterex in water at 30 and 43 p.p.m. consumed 4.3 and 5.8 mg. Dipterex per chick per day, respectively. This same age group receiving 89[3] and 132 p.p.m. Dipterex in the feed consumed 5.2 and 7.6 mg. Dipterex per chick per day, respectively.

CONVERSION TO DDVP AND TOXICITY.—It has been reported by a number of workers that Dipterex is converted to DDVP under alkaline conditions (Barthel *et al.* 1955, Mattson *et al.* 1955, Metcalf *et al.* 1959, Miyamoto 1959). Metcalf *et al.* (1959) found that there was a more rapid knockdown of adult flies by a Dipterex-sugar bait under alkaline conditions than under acid conditions and suggested that DDVP is responsible for the *in vivo* toxic action of Dipterex.

The toxicity of Dipterex and DDVP were compared by placing first instar house fly larvae in fresh manure containing 0.0001 mg. of these toxicants per gm. of manure. This dosage of Dipterex caused 5% mortality while the DDVP caused 63% mortality. Since DDVP appears to be more toxic to the larvae than Dipterex, the loss in toxicity of manure from chicks given alkaline tapwater solutions of Dipterex suggests the possibility that although Dipterex is converted *in vitro* to DDVP, the latter is metabolized by the chick and excreted as a less toxic material. This line of reasoning is further supported by comparing the toxicity of manures from chicks on diets of Dipterex-treated feeds with that of manures from DDVP-treated feed. Sherman & Ross (1960) reported that droppings from chicks on a regimen of feed containing 89 p.p.m. of Dipterex caused 94.7% mortality in first instar house fly larvae. However, DDVP added to feed in a similar manner but at a level of 220 p.p.m. resulted in droppings which caused only 59.3% mortality (Sherman & Ross, unpublished data). Thus, it appears that the chick is able to metabolize Dipterex and excrete a highly larvicidal product but when conversion to DDVP occurs *in vitro* under alkaline conditions, the metabolic pathway in the chick is different and results in products having low larvicidal activity.

AGE OF MAGGOTS AND TOXICITY.—An additional experiment was conducted to determine the effect of age of house fly larvae on susceptibility to manure from chicks on the Dipterex-acidified water solutions. The method

[3] These chicks actually received 89 p.p.m. Dipterex at 4 to 5 weeks of age but for purposes of comparison, the intake of feed was assumed to be the same as that consumed by the birds given 132 p.p.m. Dipterex in the feed at 5 to 6 weeks.

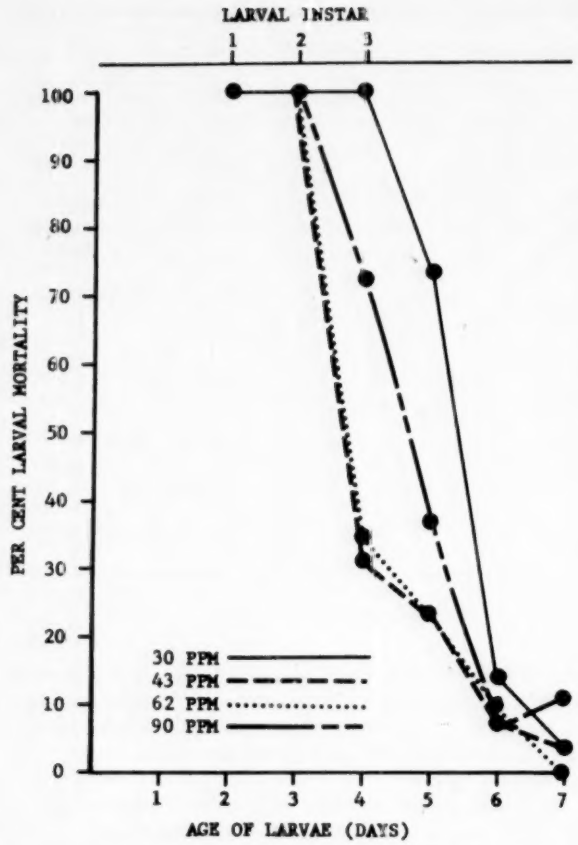

FIG. 3.—Effect of age of house fly larvae on toxicity of droppings from chicks given various concentrations of acidified tapwater solutions of Dipterex. Larval mortality corrected for natural mortality.

utilized in determining the larval mortality was the same as described earlier except for the ages of the test insect. Triplicated trials were made with 2-, 3-, 4-, 5-, 6-, and 7-day-old larvae. Prior to placement in the manure, the larvae were reared to the required age on an insecticide-free medium. The results are given in figure 3. All larvae 3 days of age and younger when placed in the manure from Dipterex-treated chicks died within 3 days. With larvae older than 3 days, the survival rate increased. The greater toxicity to larvae of droppings from chicks on the diet of 30 p.p.m. of Dipterex was probably owing to the fact that the manure resulting from the intake of this concentration was less repellent to the larvae than the manure resulting from the higher concentrations. Consequently, the larvae placed in the droppings from the chicks on the higher concentrations stopped feeding and since they were already in the third instar, pupated or died after the 3-day period of the experiment. Adults emerging from larvae which were 4 or 5 days old at the time of placement in the manure were all small; indicative of subnormal nutrition. However, adults emerging from larvae 6 or 7 days of age at the time of placement in the manure were of normal size suggesting that little or no subsequent feeding was required for normal development.

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The Relation of Moisture Content and Temperature of Stored Grain to the Effectiveness of Grain Fumigants Under Forced Circulation[1]

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ABSTRACT

Results are given of the mortality of the adults of *Tribolium confusum* Duv. and *Sitophilus oryza* (L.) obtained when fumigated with carbon tetrachloride, hydrocyanic acid, and methyl bromide under forced circulation with no load, and in the presence of wheat and corn of 10%, 12.5%, and 15% moisture content and 50°, 70°, and 90° F. and the concentration of the fumigant under these conditions.

Under conditions of forced circulation the drop in concentration of carbon tetrachloride, hydrocyanic acid, and methyl bromide was greater in corn than in wheat, and at the higher moisture contents of corn or wheat, these effects being reflected in insect mortality. The sorption of carbon tetrachloride by corn or wheat was greatest at 50° F. and least at 90° F., while temperature had little effect on the sorption of hydrocyanic acid or methyl bromide by corn or wheat.

Adults of *Sitophilus oryza* were more tolerant to carbon tetrachloride, and hydrocyanic acid than adults of *Tribolium confusum* but the reverse was true for methyl bromide. The insects were more susceptible to the fumigants at the higher temperatures.

In the fumigation of infested bulk grain in elevator storage bins, the general practice is to apply the fumigant to the grain surface or to the grain stream as it leaves the conveyor belt to fall into the bin, and depend on natural diffusion for uniform distribution of the gas throughout the grain mass. This method requires long exposures and, depending on both the fumigant and the commodity being treated, uniform distribution is not always obtained. There is also the problem of removing the gas from the grain at the end of the exposure period. The advantages of forced circulation are many and are discussed by Cotton (1956).

The use of forced circulation of fumigants in grain elevator bins to improve distribution and increase efficiency has been practiced in Europe for many years (Monro 1956). In the United States this method was first demonstrated successfully by Phillips & Latta (1949) in the treatment of cotton seed and by Cotton & Walkden (1951) in the treatment of sorghum grain.

The possible utilization of mechanical aeration systems for the recirculation of fumigants has stimulated investigations to determine those factors affecting fumigation results under recirculation conditions such as temperature, type and moisture content of commodity, and fumigant. This paper gives the results of the mortality of the adults of *Tribolium confusum* Duv. and *Sitophilus oryza* (L.) obtained when fumigated with carbon tetrachloride, hydrocyanic acid, and methyl bromide under forced circulation with no load, and in the presence of wheat and corn of 10%, 12.5%, and 15% moisture contents, and 50°, 70°, and 90° F. and the concentrations of the fumigants under these conditions.

MATERIALS AND METHODS.—*Fumigation Chambers.*—A battery of nine gastight fumigation chambers was used in these experiments. Each chamber consisted of a galvanized metal tube 7 inches in diameter and 6 feet in length and a return duct consisting of 1½-inch-inside-diameter galvanized pipe. The chamber had a volume of 53 liters; exclusive of the return duct there was a volume of 45 liters. Depending on the commodity and its moisture content, the weight of wheat or corn required to fill the chamber to capacity varied from about 70 to 80 pounds. Air movement was supplied within the chamber by a built-in multivaned fan, and, as measured with an Alnor Velometer, was 18 cu. ft./min. when empty. In the presence of wheat and corn used in these experiments the air movement was approximately 1.2 cu. ft./min. and 1.9 cu. ft./min., respectively.

These chambers were installed in a 945-cubic-foot room in which the temperature was thermostatically controlled to ±1° F. Exhaust equipment was installed for evacuation of fumigants.

Exposure and Temperature.—The exposure period was 5 hours for each experiment and the recirculation fans were allowed to run continuously. The experiments were conducted at temperatures of 50°, 70°, and 90° F.

Fumigants.—The fumigants used were carbon tetrachloride, hydrocyanic acid, and methyl bromide. Carbon tetrachloride and hydrocyanic acid were measured as liquids onto sorbent wicks which were suspended in the return duct directly in the air stream. Methyl bromide was applied as a gas by displacement through outlets in the return duct. When high dosages, especially of carbon tetrachloride, were required for effective kill of insects, a positive pressure developed in the fumigation chamber during the evaporation period and was released. Later on during the fumigation cycle, a negative pressure was developed in the chamber owing to the sorption factor. This was also released to maintain the fumigations at atmospheric pressure. All fumigants were of technical grade.

Measurement of Gas.—Gas analysis of both carbon tetrachloride and methyl bromide was determined by the thermal-conductivity method (Gow-Mac) in which a closed system was used. To measure the high concentrations of carbon tetrachloride, the range of the thermal-conductivity instrument had to be increased by adjusting to the 10% output level, thereby increasing the range, but greatly reducing the sensitivity. Hydrocyanic acid gas was determined by the silver nitrate titration method in which either 100- or 200-ml. samples (depending on whether the concentrations were high or low) were re-

[1] Paper No. 1222, University of California Citrus Experiment Station, Riverside, California. Accepted for publication June 3, 1960.

[2] This research was conducted in part with funds from the Agricultural Marketing Service, U. S. Department of Agriculture, under contract No. 12-25-010-456.

moved by bubbling through sodium hydroxide. Gas samples were taken at ½-, 1-, 2-, and 5-hour intervals following application of the fumigant.

As the work progressed it became obvious that the Gow-Mac method of gas analysis was not suitable because of its lack of sensitivity and the interference caused by carbon dioxide, moisture, et cetera. Analysis by gas chromatography overcame these difficulties (Lindgren & Vincent 1959). With gas chromatography a 5-ml. sample taken with a glass syringe from the return duct was sufficient. The concentration measurements given in this paper are, therefore, based on the thermal-conductivity method, chemical analysis and gas chromatography.

The theoretical dosage applied is given in mg./l. based on the empty chamber. But when approximately 50% to 60% of this space is occupied by the commodity, the dosage is, in effect, doubled.

Commodity.—Whole-kernel grade No. 2 recleaned White Federation wheat and whole-kernel grade No. 2 yellow corn were used as the commodities being fumigated. Only untreated grain was used. The wheat or corn was brought up to the proper moisture content of 10%, 12.5%, or 15% before fumigation. This was done by adding water to the grain and mixing thoroughly for 5 minutes, then placing the mixed grain in a moisture-proof 50-pound container and allowing it to reach equilibrium for 1 or more weeks before using it. Moisture content was based on a Steinlite Moisture Tester.

Insects.—Insects used were 2- to 3-week-old adults of *Sitophilus oryza* and *Tribolium confusum* reared at 80° F. and 60% relative humidity (R.H.) The rice weevils were reared on whole-kernel wheat with moisture content of 12% to 14%, and the confused flour beetles on a mixture of whole-wheat flour, corn meal, wheat germ, dried milk, and brewer's yeast. Approximately 120 test insects of each species were confined in separate probes which were inserted into the center of the column of grain. Following fumigation, the test insects were held with food in 2-ounce, screened-top salve tins at 80° F. and 60% R.H. Parallel controls were set up for each experiment.

Mortality counts were made 5 days following treatment; an insect was counted alive if any movement was present. With carbon tetrachloride, especially, it was difficult to distinguish between live and dead insects because of slight movement of legs and antennae. Although insects exhibiting any movement were counted as being alive, some probably would die. Natural mortality was negligible in the controls.

RESULTS.—For any fumigant at a given moisture content of grain and a given temperature, a minimum of five points was obtained to establish a dosage-mortality curve, each point being replicated five times. Dosage-mortality curves were obtained for each insect species used. The following conclusions may be drawn from the results of concentration and of insect-mortality studies:

I. *Concentration Studies.*

A. Carbon tetrachloride (fig. 1).

1. Figure 1A (wheat) indicates that at 70° F. moisture contents of 10%, 12.5%, and 15% had similar effects on the drop in concentration of carbon tetrachloride.
2. Figure 1B (corn) indicates that at 70° F. the drop in concentration of carbon tetrachloride was greatest in the case of 15% moisture content and little difference was shown between 10% and 12.5% moisture content.
3. Results given in figure 1C indicate that at equal moisture contents the drop in concentration of carbon tetrachloride was greater in the presence of corn than in the presence of wheat. Since wheat or corn occupies 50% to 60% of the air space, the theoretical dosage applied actually would be almost 800 mg./l. In space, with the exception of the initial drop which occurred within the first 30 minutes, the concentration remained practically constant. The initial drop was due in part to release of pressure caused by evaporation of the fumigant.
4. At equal moisture contents the concentration of carbon tetrachloride (fig. 1D) at the sampling intervals was, in all cases, lower at 50° than at 70° F., and lower at 70° than at 90° F. The high dosages of carbon tetrachloride used were close to or at the maximum weight that could exist in vapor form, especially at 50° and 70° F. Condensation and sorption may be contributing factors in the drop in concentration depending on the fumigation temperature.

B. Hydrocyanic acid (fig. 2).

1. Figures 2A (wheat) and 2B (corn) indicate that the drop in concentration of hydrocyanic acid is greater at 12.5% moisture content than at 10%, and greater at 15% than at 12.5%.
2. Results given in figure 2C indicate that the drop in concentration of hydrocyanic acid was greater in the presence of corn than in the presence of wheat at equal moisture contents. In space, with the exception of the initial drop, which occurred within the first 30 minutes, the concentration remained practically constant. Since wheat or corn occupies 50% to 60% of the space, the theoretical dosage applied would actually be almost 150 mg./l. A rapid drop in concentration of hydrocyanic acid in wheat or corn occurred between the time of application and the first sampling period.
3. The results (fig. 2D) indicate that temperatures of 50°, 70°, and 90° F. had little effect on the concentration of hydrocyanic acid obtained at equal moisture contents.

C. Methyl bromide (fig. 3).

1. Figures 3A (wheat) and 3B (corn) indicate that the drop in concentration of methyl bromide is greater at 12.5% moisture content than at 10% and greater at 15% than at 12.5%. It is noted in figure 3A that for wheat of 10% moisture content the concentration at the 30-minute interval was higher than the dosage applied. Since wheat occupies approximately 50% of the space, the theoretical concentration in the space is approximately twice that shown and in this particular case sorption did not overcome this theoretical concentration.
2. Results given in figure 3C indicate that the

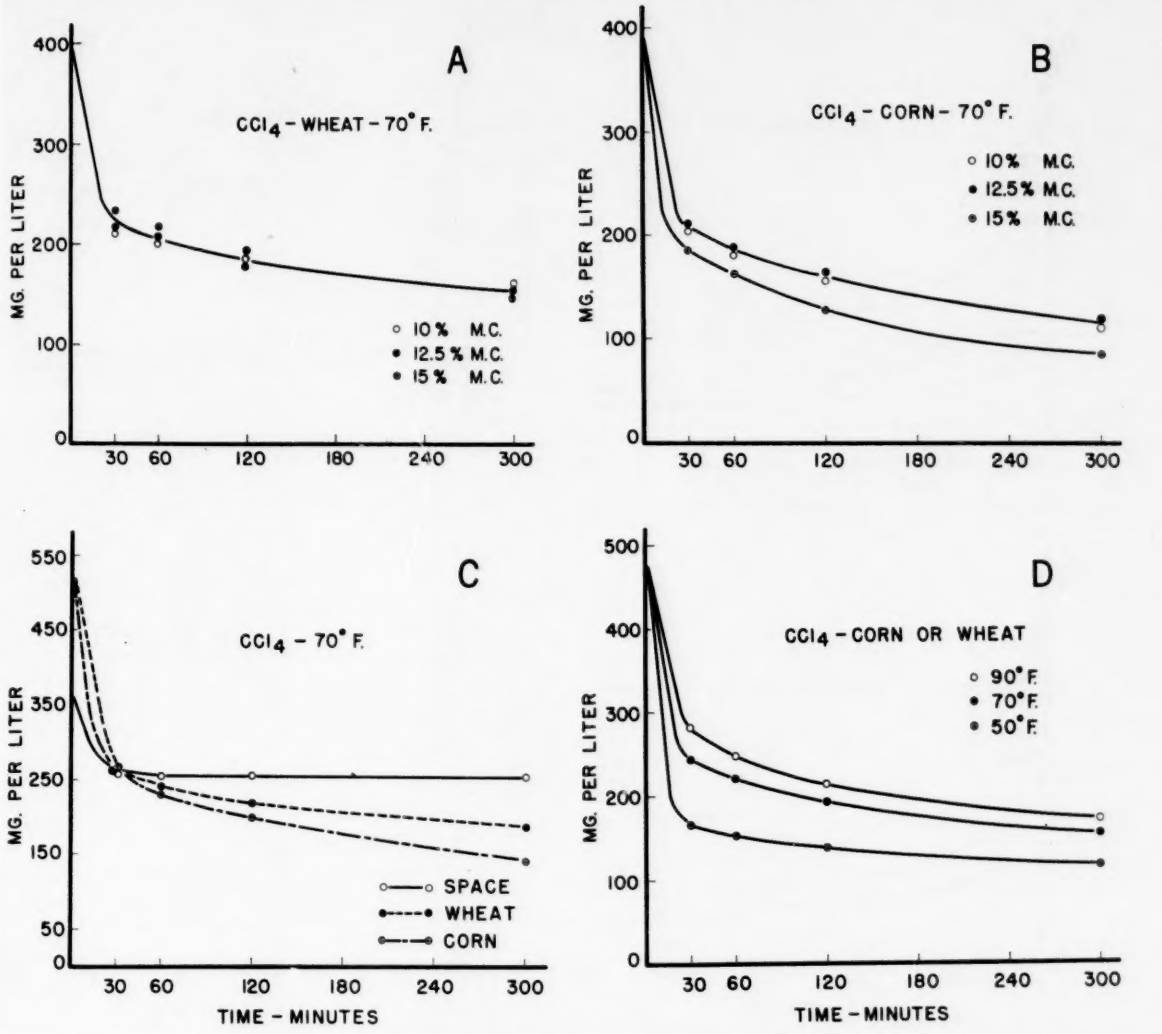

FIG. 1.—Concentration of carbon tetrachloride obtained under forced-circulation fumigation of (A) wheat, (B) corn, (C) space and corn or wheat at equal moisture contents, and (D) wheat and corn at equal moisture contents.

drop in concentration of methyl bromide was greater in the presence of corn than in the presence of wheat at equal moisture contents. In space, with the exception of the initial drop which occurred within the first 30 minutes, the concentration remained practically constant. Since wheat or corn occupies 50% to 60% of the space, the theoretical dosage applied would actually be almost 25 mg./l. In comparing the three fumigants (figs. 1C, 2C, and 3C) it will be noticed that the drop in concentration was always greater for corn than for wheat.

3. The results (fig. 3D) indicate that temperatures of 50°, 70°, and 90° F. had little effect on the concentration of methyl bromide obtained at equal moisture contents.

II. *Insect Mortality.*

A. Carbon tetrachloride (table 1-A).

1. No load.

a. Where comparisons can be made, considerably more carbon tetrachloride was required to kill 50% or 95% of *Sitophilus oryza* adults than to kill *Tribolium confusum* adults at 50°, 70°, and 90° F.

b. Under conditions of no load almost twice as much carbon tetrachloride was required to kill 95% of adults of *T. confusum* at 70° as at 90° F., and more than five times as much at 50° as at 90° F.

c. The dosages required to kill 95% of adults of *S. oryza* were greater than the amounts used.

2. Load.

a. Where comparisons at equal temperatures can be made, at LD_{50} or LD_{95} more carbon tetrachloride was required in the presence of wheat or of corn than in an empty chamber.

b. Where comparisons at equal temperatures can be made there was little difference in the amount of carbon tetrachloride required to

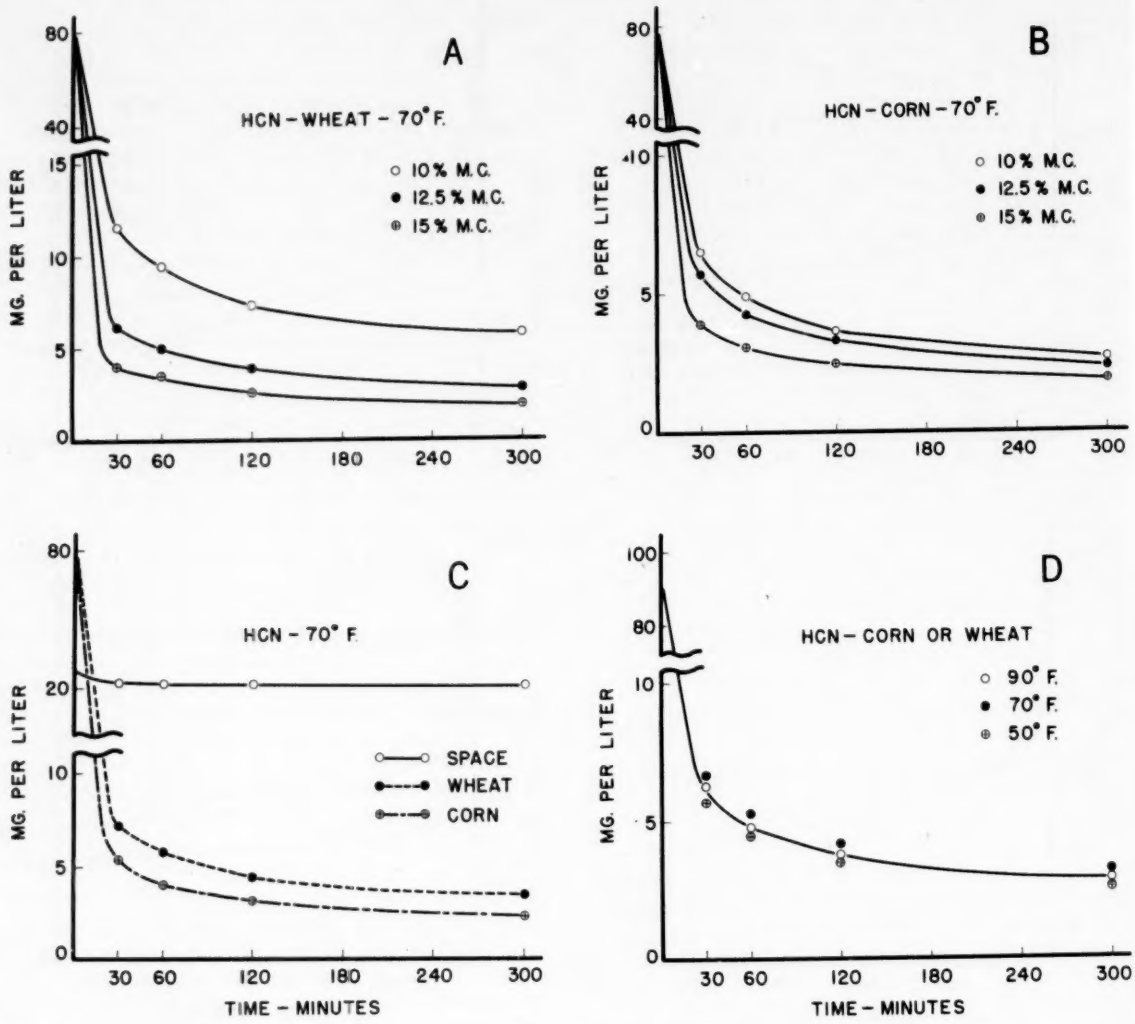

FIG. 2.—Concentration of hydrocyanic acid obtained under forced-circulation fumigation of (A) wheat, (B) corn, (C) space and corn or wheat at equal moisture contents, and (D) wheat and corn at equal moisture contents.

obtain LD_{95} at moisture contents of 10%, 12.5%, or 15%.

c. Where comparisons at equal temperatures and moisture contents can be made, at LD_{50} or LD_{95} considerably more carbon tetrachloride was needed in corn than in wheat. This difference at LD_{95} was as great as five times the dose for wheat.

d. Where comparisons at equal moisture contents can be made, at LD_{50} or LD_{95} considerably more carbon tetrachloride was required at 70° than at 90° F., and more at 50° than at 70° F.

B. Hydrocyanic acid (table 1-B).

1. No load.

a. Under conditions of no load, *S. oryza* adults are much more difficult to kill at 50°, 70°, and 90° F. than *T. confusum* adults. To obtain 95% kill of *S. oryza* adults required from 13 to 30 times as much hydrocyanic acid as for *T. confusum* adults, at equal temperatures.

b. Under conditions of no load there was little difference in the amount of hydrocyanic acid required to kill either 50% or 95% of *T. confusum* adults at 50°, 70°, or 90° F. This may be owing to the difficulty of measuring the small amounts of hydrocyanic acid required to kill *T. confusum* adults.

c. To obtain LD_{95} of *S. oryza* adults under conditions of no load required approximately twice as much hydrocyanic acid at 50° F. as at 90° F.

2. Load.

a. In every case at equal temperatures, at the LD_{50} or LD_{95} more hydrocyanic acid was required in the presence of wheat or of corn than in an empty chamber. Depending on temperature and moisture content, this difference was as great as 20 times the smaller dose at LD_{95}.

b. At the LD_{50} or LD_{95} for both *S. oryza* and *T. confusum* adults, the higher the moisture content of wheat or corn the greater the

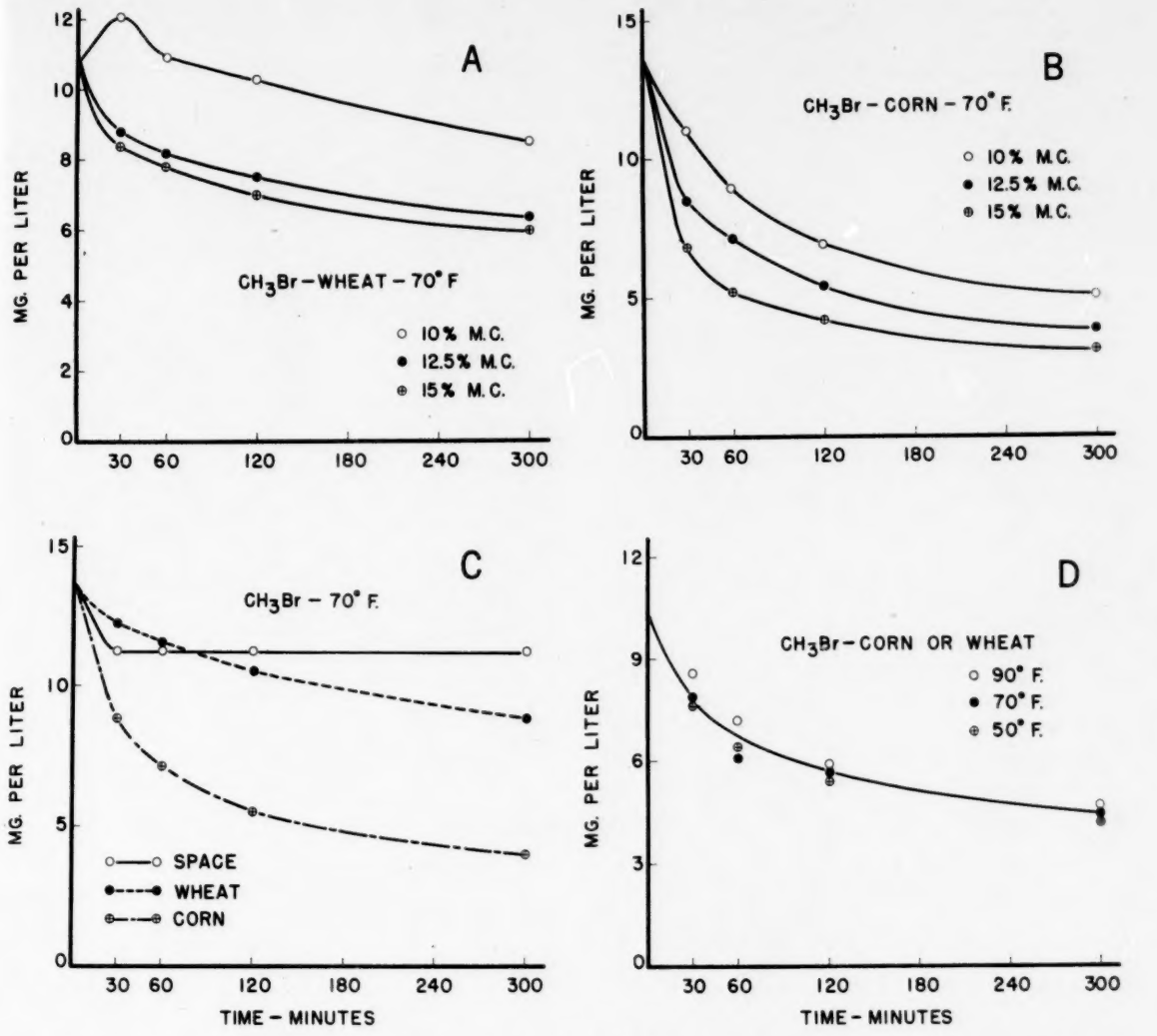

FIG. 3.—Concentration of methyl bromide obtained under forced-circulation fumigation of (A) wheat, (B) corn, (C) space and corn or wheat at equal moisture contents, and (D) wheat and corn at equal moisture contents.

dosage of hydrocyanic acid required, at equal temperatures.

c. To obtain LD_{50} or LD_{95} of *T. confusum* adults there was little difference in the amount of hydrocyanic acid required for corn or wheat. To obtain LD_{95} of *S. oryza* adults at equal temperatures and moisture contents required more hydrocyanic acid for corn than for wheat in all cases.

d. At the LD_{95} for both *S. oryza* and *T. confusum* adults, in most cases the lower the temperature at equal moisture contents the greater the amount of hydrocyanic acid required, although in many cases these differences were small. However, at LD_{50} the effect of temperature is not consistent.

C. Methyl bromide (table 1-C).

1. No load.

a. Under conditions of no load, *T. confusum* adults are more difficult to kill at 50°, 70°, and 90° F. than *S. oryza* adults. To obtain 95% kill of *T. confusum* adults required from 1.5 to 3 times as much methyl bromide as for *S. oryza* adults at equal temperatures.

b. Under conditions of no load twice as much methyl bromide was required to kill 95% of adults of *T. confusum* at 70° than at 90° F., and 2.5 times as much at 50° than at 70° F.

c. To obtain LD_{50} or LD_{95} under conditions of no load there was little difference in the amount of methyl bromide required to kill adults of *S. oryza* at 70° and at 90° F., but approximately 2.5 times as much was required at 50° as at 70° F.

2. Load.

a. At equal temperatures there was little difference in the amount of methyl bromide required to kill 50% or 95% of the adults of *T. confusum* or *S. oryza* in the presence of wheat of 10% moisture content and in an empty chamber. In general, slightly more

Table 1.—Dosage of three fumigants applied, under forced-circulation fumigation, to obtain the LD_{50} and LD_{95} of *Tribolium confusum* and *Sitophilus oryza* adults at temperatures of 50°, 70°, and 90° F. in space and in the presence of wheat and corn at moisture contents of 10%, 12.5%, and 15%.

Load and Moisture Content	Temperature (° F.)	Dosage Applied							
		T. confusum				*S. oryza*			
		LD_{50}		LD_{95}		LD_{50}		LD_{95}	
		Mg./l.	Lb./1,000 Cu. Ft.	Mg./l.	Lb./1,000 Cu. Ft.	Mg./l.	Lb./1,000 Cu. Ft.	Mg./l.	Lb./1,000 Cu. Ft.
		A. Carbon tetrachloride							
	90	20.0	1.25	74.7	4.66	134.5	8.4	> 448.0	>27.97
No load	70	47.8	2.98	119.6	7.47	621.9	38.82	> 718.0	>44.82
	50	158.5	9.9	403.7	25.2	> 598.0	>37.33	> 598.0	>37.33
Wheat	90	50.8	3.17	83.7	5.22	200.3	12.50	> 598.0	>37.33
10%	70	149.5	9.33	359.0	22.41	798.3	49.83	>1,077.0	>67.23
	50	420.0	26.22	>1,077.0	>67.23	>1,077.0	>67.23	>1,077.0	>67.23
Wheat	90	56.8	3.55	98.7	6.16	224.2	14.00	648.8	40.50
12.5%	70	194.4	12.13	359.0	22.41	882.1	55.06	>1,077.0	>67.23
	50	450.0	28.1	>1,077.0	>67.23	>1,077.0	>67.23	>1,077.0	>67.23
Wheat	90	74.7	4.66	137.5	8.58	284.0	17.73	837.0	52.25
15%	70	185.4	11.57	398.0	24.84	888.0	55.43	>1,077.0	>67.23
	50	762.5	47.60	>1,077.0	>67.23	>1,077.0	>67.23	>1,077.0	>67.23
Corn	90	101.7	6.35	433.6	27.07	334.9	20.91	998.7	62.34
10%	70	200.0	12.48	801.3	50.02	>1,077.0	>67.23	>1,077.0	>67.23
	50	852.2	53.20	>1,077.0	>67.23	>1,077.0	>67.23	>1,077.0	>67.23
Corn	90	248.2	15.5	463.5	28.93	334.9	20.91	998.7	62.34
12.5%	70	320.0	19.98	897.0	55.99	>1,077.0	>67.23	>1,077.0	>67.23
	50	1,061.5	66.26	>1,077.0	>67.23	>1,077.0	>67.23	>1,077.0	>67.23
Corn	90	209.3	13.06	478.4	29.86	388.7	24.26	941.9	58.8
15%	70	328.9	20.53	900.0	56.18	>1,077.0	>67.23	>1,077.0	>67.23
	50	950.8	59.4	>1,077.0	>67.23	>1,077.0	>67.23	>1,077.0	>67.23
		B. Hydrocyanic acid							
	90	0.8	0.05	1.2	0.07	7.0	0.44	15.5	0.97
No load	70	1.0	0.06	1.4	0.09	18.3	1.14	26.6	1.66
	50	0.6	0.04	1.0	0.06	2.5	0.16	30.3	1.89
Wheat	90	2.3	0.14	3.1	0.19	61.9	3.86	161.2	10.06
10%	70	1.9	0.12	4.1	0.26	60.6	3.78	154.8	9.66
	50	3.1	0.19	8.1	0.51	15.5	0.97	165.1	10.31
Wheat	90	3.7	0.23	5.9	0.37	96.7	6.04	181.0	11.30
12.5%	70	3.6	0.22	5.4	0.34	122.6	7.65	267.0	16.67
	50	4.9	0.31	10.8	0.67	45.2	2.82	335.4	20.94
Wheat	90	5.0	0.31	7.1	0.44	116.0	7.24	284.0	17.73
15%	70	5.2	0.32	7.7	0.48	161.3	10.07	354.8	22.15
	50	7.9	0.49	20.6	1.29	51.6	3.22	412.8	25.77
Corn	90	2.3	0.14	4.3	0.27	135.5	8.46	228.3	14.25
10%	70	2.8	0.17	4.3	0.27	103.0	6.43	270.9	16.91
	50	5.2	0.32	9.9	0.62	59.3	3.70	516.0	32.21
Corn	90	4.6	0.29	5.4	0.34	154.8	9.66	258.0	16.10
12.5%	70	3.1	0.19	6.7	0.42	108.4	6.77	374.1	23.35
	50	7.7	0.48	16.8	1.05	81.3	5.07	554.7	34.63
Corn	90	5.8	0.36	7.4	0.46	187.1	11.68	341.9	21.34
15%	70	4.1	0.26	8.1	0.51	154.8	9.66	438.6	27.38
	50	7.7	0.48	17.4	1.09	219.3	13.69	670.8	41.87
		C. Methyl bromide							
	90	5.6	0.35	6.6	0.41	3.1	0.19	4.1	0.26
No load	70	12.2	0.76	14.1	0.88	3.5	0.22	4.6	0.29
	50	26.6	1.66	35.0	2.18	7.1	0.44	11.2	0.70

Table 1.—(Continued)

Load and Moisture Content	Temperature (° F.)	Dosage Applied							
		T. confusum				*S. oryza*			
		LD_{50}		LD_{95}		LD_{50}		LD_{95}	
		Mg./l.	Lb./1,000 Cu. Ft.	Mg./l.	Lb./1,000 Cu. Ft.	Mg./l.	Lb./1,000 Cu. Ft.	Mg./l.	Lb./1,000 Cu. Ft.
Wheat 10%	90	5.2	0.32	6.3	0.39	3.5	0.22	4.1	0.26
	70	10.8	0.67	13.2	0.82	3.6	0.22	4.9	0.31
	50	21.7	1.35	28.2	1.76	7.7	0.48	11.4	0.71
Wheat 12.5%	90	6.2	0.39	6.5	0.41	3.6	0.22	4.5	0.28
	70	13.3	0.83	15.5	0.97	4.3	0.27	5.5	0.34
	50	32.2	2.01	45.9	2.87	10.2	0.64	16.1	1.00
Wheat 15%	90	7.1	0.44	7.6	0.47	3.9	0.24	4.9	0.31
	70	15.4	0.96	17.9	1.12	4.6	0.29	6.4	0.40
	50	37.2	2.32	49.0	3.06	10.3	0.64	17.2	1.07
Corn 10%	90	8.4	0.52	9.8	0.61	4.8	0.30	6.0	0.37
	70	17.9	1.12	20.5	1.28	6.0	0.37	8.6	0.54
	50	38.7	2.42	52.2	3.26	11.6	0.72	18.6	1.16
Corn 12.5%	90	10.0	0.62	11.4	0.71	5.7	0.36	7.2	0.45
	70	18.2	1.14	21.6	1.35	6.5	0.41	9.1	0.57
	50	46.3	2.89	57.4	3.58	15.5	0.97	22.4	1.40
Corn 15%	90	12.1	0.76	13.6	0.85	6.4	0.40	8.0	0.50
	70	22.6	1.41	30.5	1.90	8.3	0.52	10.2	0.64
	50	51.2	3.20	75.6	4.72	17.4	1.09	25.9	1.62

methyl bromide was required to kill 50% or 95% of the adults of *T. confusum* and *S. oryza* in the presence of wheat of 12.5% moisture content than in an empty chamber. At equal temperatures more methyl bromide was required to kill adults of *T. confusum* and *S. oryza* in the presence of wheat of 15% moisture content than in an empty chamber.

Every case at the LD_{50} or LD_{95} required more methyl bromide in the presence of corn than in an empty chamber, at equal temperatures.

b. At the LD_{50} or LD_{95} for *T. confusum* and *S. oryza* adults, the higher the moisture content of either wheat or corn, the greater the dosage of methyl bromide required, at equal temperatures.

c. To obtain LD_{50} or LD_{95} of *T. confusum* and *S. oryza* adults required more methyl bromide in all cases in corn than in wheat, at equal temperatures and moisture contents.

d. At the LD_{50} or LD_{95} for both *T. confusum* and *S. oryza* adults, the lower the temperature the greater the amount of methyl bromide required at equal moisture contents.

To kill 95% of *S. oryza* adults in the presence of either corn or wheat at equal moisture contents required approximately three times as much methyl bromide at 50° as at 90° F., while *T. confusum* adults required at least 4.5 times as much methyl bromide at 50° as at 90° F. to obtain LD_{95}.

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Sampling Experimental Plots of Alfalfa for Insecticide Residues[1]

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Abstract

A procedure combining both random and systematic methods of sampling was developed for sampling experimental plots of alfalfa for insecticide residues. It was found that a single chemical analysis made from a 20-composite sample taken from a 20- by 40-foot plot was about ideal for determining insecticide residues on alfalfa. The procedures and statistical methods are outlined in detail.

According to Federer (1955), "Sampling procedures such as the stratified-random and the systematic are usually more efficient than samples selected completely at random from the entire plot." A sampling method utilizing a stratified-random and systematic sampling procedure was developed in consultation with Professor Robson of Cornell University. Utilizing this new sampling procedure in the summer of 1959, an experiment was designed to determine the optimum number of samples and the optimum plot size for insecticide residue experiments on alfalfa.

Materials and Methods.—A uniformity trial consisting of 4 blocks of 6 plots each was conducted on a level field with a uniform stand of first-cutting DuPuits variety alfalfa which averaged 8 to 10 inches high. Methoxychlor was applied as emulsion at the rate of 1 pound of actual toxicant per acre in 20 gallons of water at 40 p.s.i. pressure with a "Yellow Devil," low-pressure, low-volume sprayer equipped with 11 no. 8002 T-Jet nozzles on a 20-foot, brush boom.

Each of the 6 plots in each block was 20 feet wide by 80 feet long. Since the experiment was designed to compare the variation in plots of 20 by 20, 20 by 40, 20 by 60, and 20 by 80 feet, each plot was subdivided into 20, 40, and 60 foot lengths. The same subdivision was used in each plot within a block. The width of plot, 20 feet, was chosen on the basis of the practical consideration that the equipment used for residue tests at this station and by most growers in this area has a swath width of 20 feet. Since every effort was made to obtain experimental conditions similar to those used by alfalfa growers, this width was deemed the most desirable width to use for plots in the residue experiments. Also, the lengths to sample which were chosen were those considered the most practical. The maximum length of 80 feet was chosen because the short, rolling nature of many fields in this area is such that a uniform replicate is often hard to fit on the field if the plots are much over 80 feet in length. For further details concerning experimental design see Huddleston (1960).

The sampling rate of 5, 10, 15, and 20 areas per plot size were chosen on the basis of previous experiments with random sampling (Huddleston & Gyrisco 1958). As a further consideration, 40 areas were sampled in each 20- × 40-foot plot and duplicate samples of each of the four main rates of sampling were taken also from the same series of plots. The sampling rate of 40 areas per plot was included in the experiment in order to have an indication of the amount of variation beyond the largest rate, but was not used for all plot sizes because of limitations on the number of samples that could be analyzed. Duplicate samples were taken to give an indication of the relative error due to sampling as opposed to the remainder of the experimental error.

Sampling of the plots was begun 16 days after treatment and completed on the evening of the following day. The 2-week plus interval following treatment was chosen as the time of sampling because it is about midway between 2 and 3 weeks after application—the two harvest dates suggested most frequently on insecticide labels.

The sampling method used in this experiment was a combination of random, stratified, and systematic sampling. Buffer strips 2.5 feet wide were removed from each side of each plot and the remaining 15-foot area was divided into 3 subplots (A, B, & C) each 5 feet wide.

For example, the distance used in systematic spacing (in the 20- ×40-foot plots) of sampling stations was obtained by dividing the sum total length of the three 40-foot subplots (120 ft.) by the optimum number of samples, 20 (Huddleston & Gyrisco 1958) which gave 6 feet. Each subsampling station, therefore, was 6 feet from the preceding one. The location of the first sampling area, for example 2 feet, from the first 6 feet of subplot A for each plot was chosen at random using a table of random numbers. (Since the last subsampling station in subplot A (in the example) was 2 feet from the end, the first subsampling station in subplot B was 4 feet from that end of subplot B.) The actual area sampled was chosen at random from the imaginary line across the subplot at the systematically previously chosen sampling belts.

At the site finally chosen for the subsample, 1 to 2 square-foot areas of alfalfa were cut, mixed, and one-twentieth of the desired composite sample weight (500 grams) taken. Use of this technique assured that each sampling site was equally represented in the final composite sample and that the leaves and stems were in their true proportions. After weighing several subsamples, the experimenters were able to select composite samples which weighed reasonably near the desired (500 gm.) weight. While the weight of foliage from each area did vary to a certain degree, this composite method required little or no subdivision of the final composite sample and the variation in weight from area to area was therefore random which contributed no identifiable bias toward the sample result.

The actual sampling method used in the uniformity trial was a modification of a method developed by the authors from Cornell University and T.W. Kerr and C.E. Olney of the University of Rhode Island in a coopera-

[1] Accepted for publication June 13, 1960.
[2] This work was supported in part by funds from the Northeastern Regional Project NE-36.
The authors gratefully acknowledge the assistance of S. W. Jacklin, R. L. Ridgway, C. S. Koehler, H. H. Shorey, R. J. Little, Jr. and H. Y. Forsythe, Jr. in some phases of the work.

tive study of the previous year (Huddleston & Gyrisco 1958). Since a large number of areas were sampled from each plot, the entire plot was mowed, a third at a time, lengthwise. The location of the areas to be sampled, previously chosen, were written on the paper bag for each sample. With a tape stretched on the mowed foliage, the foliage from each area could be placed in the correct bag and one-third of each sample could be taken before the next third of the plot was mowed. Thus plot variation due to drying of the alfalfa was held to a minimum. At the end of each day, the samples were placed in plastic bags in a freezer until extraction. Weights were taken at the time of extraction. The standard extraction procedure and the modified Schecter method (Schecter *et al.* 1945) of chemical analysis for methoxychlor were used. The data obtained by chemical analysis are expressed as parts per million of methoxychlor on a green weight basis.

DISCUSSION AND RESULTS.—The analysis of variance in table 1 is based on the following experiment: A 4^2 factional treatment arrangement (4 plot sizes and 4 sampling rates) was applied at random in each of 6 plots in each of 4 blocks. The analysis is exactly analogous to that of the one appearing in Federer (1955), page 302.

The sources of variation were divided into that due to replicates, treatments, and experimental error. The large mean square for replicates indicates a much larger difference between blocks than was expected from an observation of the level field. These data clearly indicate the need for blocking and replication in any residue experiment. A part of the replicate sum of squares was due to the order in which samples were extracted and analyzed. Each sampling rate in each replicate was handled as a unit in both procedures.

The treatment mean square was not significantly larger than the error mean square. However, this result is to be expected in a uniformity trial since each plot was treated alike and each treatment value was an estimate of the same value or plot mean. The division of the treatment sum of squares into the three components indicates a difference due to numbers of samples. The explanation of this difference lies partly in the experimental procedure already discussed. Since all samples for one sampling rate were handled as a unit, any variation in sample weight due to handling of samples between harvest and extraction and any variation in unit to unit in the chemical analysis would appear here. The small sum of squares for plot sizes would indicate that this statement is true since plot sizes were evenly distributed throughout each extractio and sampling unit.

Table 1.—Analysis of variance of data from a factorial analysis of four plot sizes and four sampling rates with sampling by the stratified-systematic-random sampling procedure. 1959.

SOURCES OF VARIATION	D.F.	SUM OF SQUARES	MEAN SQUARE
Replicates	3	1,090.60	363.54
Treatments	15	63.85	4.26
Plot size	3	10.79	3.60
Sample size	3	32.02	10.68
Plot size×sample size	9	21.02	2.34
Treatments×Replicates	45	233.84	5.20
Plot size×replicates	9	27.74	3.08
Sample size×replicates	9	145.41	16.16
Plot size×sample size ×replicates	27	50.68	1.88
Error	314	1,028.03	3.28

Table 2.—Partition of the error sum of squares from table 1 into the contribution of each sampling rate, with additional partition of each sampling rate, error sum of squares into the contribution of each plot size. 1959.

COMPONENT OF ERROR SUM OF SQUARES	D.F.	SUM OF SQUARES	MEAN SQUARE
5 samples per plot	77	436.61	5.67
per 20′×20′ plot	19[a]	118.56	6.24
per 20′×40′ plot	19[a]	97.97	5.16
per 20′×60′ plot	19[a]	104.21	5.48
per 20′×80′ plot	20	115.87	5.79
10 samples per plot	80	243.87	3.05
per 20′×20′ plot	20	55.06	2.75
per 20′×40′ plot	20	40.91	2.05
per 20′×60′ plot	20	93.17	4.66
per 20′×80′ plot	20	54.73	2.74
15 samples per plot	79	189.37	2.40
per 20′×20′ plot	20	56.55	2.83
per 20′×40′ plot	19[a]	42.58	2.24
per 20′×60′ plot	20	39.34	1.97
per 20′×80′ plot	20	50.90	2.55
20 samples per plot	78	158.16	2.03
per 20′×20′ plot	19[a]	35.47	1.87
per 20′×40′ plot	20	47.50	2.38
per 20′×60′ plot	19[a]	48.21	2.54
per 20′×80′ plot	20	26.98	1.35

[a] Less 1 df for missing plot.

The nonsignificant treatment times replicate mean square is an indication that the response of treatments did not change from replicate to replicate. Apparently the large sampling rate by replicate interaction is related to the large sampling rate sum of squares and is explained on the same basis.

The error sum of squares is of major interest in a uniformity trial of this nature as it is the sum of squares which must be reduced by adequate sampling and the refinement of other experimental techniques. The size of the error term is proportional to the power of the experiment to detect differences which exist between treatments. Since each sampling rate and each plot size was expected to have a different error variance, the error sum of squares was partitioned in two different ways (tables 2 and 3). The contribution of each of the 16 treatments to the error sum of squares was obtained by a simple analysis of variance of each treatment. The total sum of squares for each treatment was divided into that due to replicates and to the residual or error sum of squares. Without the use of complicated formulae, the proof of this calculation is found by adding the error sum of squares for each treatment. The total equals the error sum of squares for each treatment and the average variance for each sampling rate. Table 3 gives the same values arranged by plot size with the average variance given for each plot size.

The comparison of the variance of the different sampling numbers yields the information for which this experiment was designed. As the number of areas sampled per plot increases, the variance of the observations decreases. The rate of decline of the variance did not diminish as rapidly as in the previous study. Because of this, the variance of samples of 40 areas per plot were computed to be 2.13. These data indicated that little or no decrease in variation occurred when the sampling rate was doubled from 20 to 40 areas per plot.

Table 3.—Partition of the error sum of squares from table 1 into the contribution of each plot size, with additional partition of each plot size sum of squares into the contribution of each sampling rate. 1959.

Component of Error Sum of Squares	D.F.	Sum of Squares	Mean Square
20′×20′ plots	78	265.64	3.41
5 samples per plot	19[a]	118.56	6.24
10 samples per plot	20	55.06	2.75
15 samples per plot	20	56.55	2.83
20 samples per plot	19[a]	35.47	1.87
20′×40′ plots	78	228.96	2.94
5 samples per plot	19[a]	97.97	5.16
10 samples per plot	20	40.91	2.05
15 samples per plot	19[a]	42.58	2.24
20 samples per plot	20	47.50	2.58
20′×60′ plots	78	285.03	3.65
5 samples per plot	19[a]	104.21	5.48
10 samples per plot	20	93.17	4.66
15 samples per plot	20	39.34	1.97
20 samples per plot	19[a]	48.21	2.54
20′×80′ plots	80	248.48	3.11
5 samples per plot	20	115.87	5.79
10 samples per plot	20	54.73	2.74
15 samples per plot	20	50.90	2.55
20 samples per plot	20	26.98	1.35

[a] Less 1 df for missing plot.

Also, from the analysis of variance of duplicate samples taken from all 20- by 40-foot plots, there appeared to be little or no value in using a sampling rate of more than 20 areas per plot. Sampling 20 areas per plot apparently reduced the sampling error to an optimum level.

A comparison of the average variance for each plot size indicated little or no difference due to plot size, thus, all plot sizes used may be fairly equal in efficiency.

The conclusions as to the optimum plot size and sampling number to be used are to use a 20- by 40-foot plot and sample 20 areas per plot. The choice of the sampling rate is based on the assumption that the very negligible cost of sampling additional areas in relation to the high cost of chemical analysis means that the extra time used in sampling more areas yields the most efficient estimate of the plot mean. The choice of a plot size is somewhat arbitrary. First, 20- by 40-foot plots have been the standard size used here in the past and have proven to be the most convenient. Second, information was available for more than 20 samples and for duplicate samples from this plot size.

On the basis of this experiment, the 20- by 40-foot plot size and a single sample of 20 composite subsamples will be chosen for use here in future residue sampling experiments on alfalfa. The subsamples will be chosen at random from a stratified, systematic scheme.

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The Persistence of Trithion, an Organophosphorus Insecticide, in Soil[1]

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Abstract

Studies were conducted to determine the persistence of Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) in three California loamy soils.

The influence of partial soil sterilization, fumigation, Trithion formulation, and dosage on the rate of degradation in soil was evaluated.

Trithion residues were determined by a cholinesterase inhibition method.

The persistence of Trithion increased significantly in soil which was autoclaved or fumigated with Vapam® (sodium *N*-methyl dithiocarbamate). Its longer persistence in autoclaved and fumigated soil apparently resulted from a partial destruction of the soil microorganisms which may prevent degradation of Trithion.

Considerable work has been done in the past several years on the persistence of chlorinated hydrocarbon insecticides in soils. Among the workers in this field, Lichtenstein & Polivka (1959) have shown that applications of chlordane, BHC, heptachlor, and aldrin to turf soils resulted in residues ranging from 0% to 20% of the applied dosage, 12 to 4 years after soil treatment. Boswell *et al.* (1955) reported that DDT dissipated slowly in a sandy loam soil and that between 90% and 100% was recovered from the soil 1½ years after treatment. These workers also found high soil residues of BHC, chlordane and toxaphene.

Recently, nonsystemic organophosphorus insecticides have gained wider usage as soil insecticides because of the fact that they show good control of several species of soil insects which have developed resistance to the chlorinated hydrocarbon insecticides.

Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) is one of these new, promising soil insecticides. Howitt (1958) showed that the application of Trithion as a furrow treatment gave excellent control of the onion maggot, *Hylemya antiqua* (Meig.), in the state of Washington. Miller (1959) reported that 5% granulated Trithion, at the rate of 1 pound active per acre, was very effective in controlling the onion maggot

[1] Partial cost of publication of this paper was met by the Stauffer Chemical Co. Accepted for publication September 6, 1960.

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[4] The authors wish to acknowledge the assistance of R. Curtis, Agricultural Research Laboratory, Mountain View, in determining the physical characteristics of the soils and of B. J. Adelson, Research Laboratory, Richmond, on the analytical work.

in southwestern Ontario, Canada. In general, Trithion proved to be an effective soil insecticide in several areas of North America. In the present investigation the following factors were studied: formulation, rate of application, soil type, and soil microorganisms.

MATERIALS AND METHODS.—Trithion either a 40- to 60-mesh granular clay formulation containing 5% W or an aqueous emulsion containing 4 pounds of active ingredient per gallon, was added to the soil at the rate of 1 part and 10 parts of technical Trithion per million of dry soil. The higher rate represents a recommended rate of application and the lower level was selected in order to compare the degradation of Trithion at reduced dosage levels.

In this study three California soil types were used: Sorrento loam, Santa Cruz loamy sand, and Yolo silty clay loam. The formulated Trithion was thoroughly mixed with the soil by tumbling in a ball mill. Soil moisture was adjusted to approximately 10% by weight by mixing chilled treated soil with crushed ice. The samples were stored at room temperature in loosely capped jars to allow contact with air and to insure a favorable environment for aerobic microorganisms in the soil. The above moisture level was maintained for the duration of the experiment by periodically adjusting the moisture content. Portions of the soil samples were analyzed for Trithion at given intervals after thorough mixing.

Soil extracts were prepared for analysis by a single extraction of 200-gm. soil samples with a solution containing 100 ml. of methanol and 200 ml. of benzene. The slurry was mixed for 1 hour on a mechanical tumbler. The soil was allowed to settle and the supernatant liquid was filtered into a separatory funnel. The alcohol was extracted from the benzene by shaking with 600 ml. of water. The benzene extract was separated from the aqueous phase and washed again with 600 ml. of water. Aliquots of the washed benzene extract were then analyzed for Trithion residues by a cholinesterase inhibition method developed by Patchett & Batchelder (1960). The method involves oxidation of Trithion and its oxidized derivatives to strong cholinesterase inhibitors by a hydrogen peroxide-acetic acid two-phase system.

RESULTS AND DISCUSSION.—The characteristics of the three soils used in these experiments are shown in table 1. Santa Cruz loamy sand is a sandy soil with a high organic matter content. Sorrento loam and Yolo silty clay loam make up a large portion of the best agricultural land in California. Both belong to the same series-group of soils, but differ in their physical composition.

Degradation of Trithion in Soils and Water.—The degradation pattern of Trithion in three soils and in distilled water, sampled at various intervals, is shown in table 2. The degradation of Trithion was fastest in Yolo silty clay loam, slowest in Sorrento loam and intermediate in Santa Cruz loamy sand. Although the organic matter content of the Sorrento and Yolo loams was nearly equal (table 1), they differed greatly in their effect on degradation of Trithion. The soil pH had no apparent effect on the persistence of Trithion, whereas it appears that the higher clay content in the Yolo silty clay loam contributed to Trithion degradation. A comparison of the degradation of Trithion in Sorrento loam and Santa Cruz loamy sand shows that the greater persistence of Trithion in Sorrento loam correlates well with the lower organic matter content.

Trithion, by reference to reported data on other organophosphorus insecticides, is relatively stable to hydrolysis. When Trithion was added to distilled water at the rate of 0.34 p.p.m., (saturation level) the time from 50% hydrolysis at 20° C. was 115 days (table 2). The time required for 50% hydrolysis at 25° C. for TEPP and parathion was 6.8 hours and 120 days, respectively (Metcalf 1955). Young (1957) reported that the time for 50% hydrolysis of phorate (Thimet®) at 20° C. was 7.2 days. The relatively slow degradation of Trithion in water with respect to other organophosphorus insecticides and its persistence in moist sterile soil tends to indicate that soil moisture *per se* has little effect on the degradation of Trithion in soil.

Persistence of Trithion in Autoclaved Soil.—The effect of partial soil sterilization on the persistence of Trithion is shown in figure 1. Trithion was added to Santa Cruz loamy sand at rate of 10 p.p.m. active ingredient using a 5% granular formulation. It was shown in table 2 that the time required for 50% degradation of Trithion in Santa Cruz sand was 170 days. In a similar experiment using autoclaved Santa Cruz sand it was shown that the time required for 50% degradation was extended to 525 days (fig. 1).

Table 1.—Some characteristics of three California soils used for degradation studies with Trithion.

Soil Type	Organic Matter (%)	pH	Physical Composition (%)		
			Sand	Silt	Clay
Sorrento loam	1.8	7.5	47	35	18
Santa Cruz loamy sand	5.0	4.6	82	13	5
Yolo silty clay loam	2.1	7.7	8	59	33

Table 2.—Stability of 5% granulated Trithion in three soils and in aqueous solution at various intervals after treatment.

Test Media	P.P.M. Residue[b] After Days Indicated				Days for 50% Degradation[c]
	0[a]	33	78	180	
Sorrento loam	10.0	9.4	7.7	4.8	200
Yolo silty clay loam	10.0	7.4	6.9	2.6	100
Santa Cruz loamy sand	10.0	9.4	8.2	4.8	170
	0	21	60	170	Days for 50% Degradation
Distilled water saturated with Trithion	0.37	0.29	0.25	0.11	115

[a] 5% granular formulation added to make 10 p.p.m. technical Trithion in the soil.
[b] Residue values represent an average of two to four analyses of soil extract. All determinations are expressed on dry weight of soil.
[c] 50% degradation values were determined from a semi-logarithmic plot of the data.

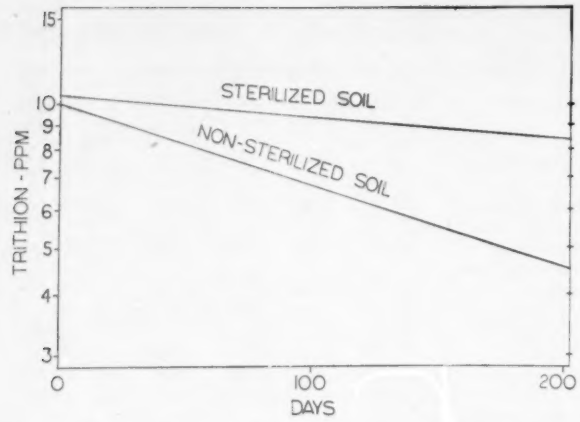

FIG. 1.—Degradation of Trithion in autoclaved and nonautoclaved Santa Cruz loamy sand.

The results indicate that partial destruction of soil microorganisms has significantly prolonged the persistence of Trithion in the soil. These findings parallel the results of Lichtenstein & Schulz (1960) who showed that prolonged autoclaving of soil for as long as 4 days only resulted in partial destruction of soil microorganisms yet contributed greatly to the increased persistence of aldrin in soil.

Influence of Chemical Soil Fumigation and Formulation on the Persistence of Trithion in Sorrento Loam.—The effect of fumigation on the persistence of Trithion in field soil was studied in Sorrento loam (table 3). Soil samples were collected from the upper 3 to 6 inches of soil from a field fumigated by drenching with Vapam® (sodium *N*-methyl dithiocarbamate) at a rate of 1 quart in 4 gallons of water per 100 square feet, 7 days prior to sampling.

Trithion, applied as a granular formulation or as an emulsion, persisted much longer in soil treated with Vapam than in untreated soil. The increased longevity in fumigated soil can best be demonstrated by comparing the relative persistence of 5% granulated Trithion in fumigated and nonfumigated soils (table 3). Granulated Trithion at the 10 p.p.m. level persisted four times longer in the fumigated soil than in the untreated soil. The Trithion emulsion at the 10 p.p.m. level also persisted longer in the Vapam-treated soil.

In table 3 it is shown that Trithion, in the granular and emulsion formulations, persisted longer in the fumigated soil at the 10 p.p.m. level than at 1 p.p.m. In the untreated soil, Trithion persisted longer at the 10 p.p.m. level in the emulsion formulation, but failed to show increased persistence at the 10 p.p.m. level of the granular formulation. As in the case of autoclaved soil (fig. 1), the increased persistence of Trithion in the field soil fumigated with Vapam could be attributed to a partial destruction of the soil microorganisms by the fumigant, resulting in the inhibition of microbial metabolism of Trithion in the soil.

CONCLUSIONS.—It was shown that Trithion degraded in soil much faster than several chlorinated hydrocarbon insecticides, and would offer seasonal control of soil-borne insect pests without leaving residues which would be objectionable in the soil for an extended period of time.

Trithion remained considerably longer in Sorrento loam than in a sandy soil and persistence was greatly increased in either autoclaved or fumigated soil. By applying Trithion to fumigated soils, much longer insect control may be achieved. The practice of soil fumigation prior to treatment with Trithion could be advantageously employed in nursery and greenhouse plantings where active residues of insecticides would be useful and no food crops are involved.

Table 3.—The effect of formulation and soil fumigation on the degradation of Trithion in Sorrento loam soil.

TRITHION TREATMENT (SOIL INCORPORATION, P.P.M.)	P.P.M. RESIDUE[a] AFTER DAYS INDICATED				DAYS FOR 50% DEGRADATION
	35	60	148	270	
Vapam fumigated soil					
Emulsion[b] 10	—	14.3	9.3	8.1	364
Granular[c] 10	—	8.2	9.1	6.9	750
Emulsion 1	—	1.7	0.76	0.59	180
Granular 1	—	1.1	0.83	0.70	345
Untreated	0.011	—	—	0.011	—
Nonfumigated soil					
Emulsion 10	—	14.6	10.0	7.4	230
Granular 10	—	10.0	7.0	4.7	190
Emulsion 1	—	1.53	0.31	0.55	140
Granular 1	1.23	—	0.67	0.52	230
Untreated	0.005	—	—	0.002	—

[a] Residue values represent an average of two to four analyses of soil extract. All determinations are expressed on dry weight of soil.
[b] Trithion emulsion—a 4 lb. per gallon aqueous emulsion.
[c] Trithion 5% on clay granules.

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DDT-Resistant Codling Moths on Pears in California[1]

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ABSTRACT

Several reports were received indicating inadequate control of codling moth (*Carpocapsa pomonella* (L.)) on pears with DDT during the 1958 season.

Field plots were established in three of the orchards to determine if a resistance problem was present and to evaluate other materials and combinations. Resistance was indicated by poor control with DDT, and no significant difference between plots treated with DDT plus parathion and parathion alone. Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) and Ethyl Guthion® (*O,O*-diethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) at 1½ pounds per 100 gallons gave excellent control. When the dosage was reduced to ¾ pound, there was a significant increase in codling moth entires in one trial but not in another. An emulsion concentrate formulation was not as effective as the wettable powder.

Sevin® (1-naphthyl methylcarbamate) provided good control in all of the trials. Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta[cd] pentalen-2-one) and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) did not provide commercial control. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) was effective when used at 2-week intervals, but was not satisfactory when compared with Sevin and Guthion in a standard schedule timed by bait pan catches.

The first instance of codling moth resistance to DDT on pears in California was observed during the 1957 season. The evidence from field trials was reported by Madsen & Hoyt (1958) and later confirmed by laboratory tests conducted by Barnes (1958).

More recently there have been increasing reports of poor control with DDT. At the end of the 1958 season, there were several reported failures with well-timed and thoroughly applied DDT programs. The reports from two pear growing districts in Sacramento and Solano counties were far removed from the previously reported resistant population in Santa Cruz County. In Sacramento County, a single orchard was involved, but in Solano there were several orchards within a radius of 5 to 6 miles.

In order to determine if DDT resistance was the problem, field trials were established in the single orchard from Sacramento County and in two orchards from Solano County. In all three orchards it was possible to obtain a past history of the use of DDT and a fairly accurate report of the percentage of wormy fruit found during the 1958 season.

MATERIALS AND METHODS.—For convenience, the three orchards will be designated as A, B, and C. Orchard A was located in Sacramento County and orchards B and C in Solano County. In orchards A and B, the 1959 plots were designed to determine if a DDT resistance was present, to test the combination of DDT and parathion against a suspected resistant strain, and to evaluate some substitute compounds under conditions of a high potential population. The other compounds were Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) wettable and emulsifiable, Ethyl Guthion® (*O,O*-diethyl *S*-(4 oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), Sevin® (1-naphthyl methylcarbamate), Kepone® (decachloroöctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one), and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate). These materials had previously been evaluated in California by Madsen & Hoyt (1958) and in Connecticut by Garman (1959).

In orchard C, the major objective was to evaluate Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) as an insecticide for codling moth control. It was used in a schedule determined by bait pans and compared with Sevin and Guthion. Diazinon was also applied at 2-week intervals and compared with malathion at the same schedule. In addition, DDT was applied to determine if a resistance was present.

In all three orchards, the plots consisted of four trees with three replications in a randomized block design. Materials were applied with conventional high pressure spray equipment using orchard spray guns. The gallonage per acre varied from 450 to 600 depending upon the size of the trees involved. The timing of treatments was determined by the use of bait pans using a brown sugar solution with terpinyl acetate as an attractant.

It is the usual case in California to have a first flight of codling moth in mid-April with the first brood emergence continuing through May and early June. In the 1959 season, however, the first moths were recorded on March 30, and it was necessary to apply the first spray in early April. The early emergence was probably caused by an unusually warm winter in which temperatures were several degrees above normal. The above normal temperatures were as follows: December 5.2°, January 4.1°, and March 3.2°. Such an influence of temperature upon the pupation of overwintering codling moth has been shown by Post & DeJong (1958).

In 1959, moths were trapped through the month of April into May, and a second application for the first brood was applied the first week of May. Following this treatment, the bait pan catches were negligible until the the second brood flight occurred in mid-June. A third application was made at this time. The pans indicated a third brood flight in late July, but this flight did not affect the crop as harvest was nearly completed by this time.

Control was evaluated by sampling the fruit from each replicate during harvest and examining it for the presence of entries and stings. Two hundred fruits were checked per replicate, making 600 per treatment. In addition, 100 dropped fruits were collected per treatment and included with the total fruit counts.

RESULTS OF THE FIELD TRIALS.—In orchard A, counts taken at the end of the 1958 season demonstrated an infestation of 92% with many fruits having more than one entry. The grower had made five applications of DDT,

[1] Accepted for publication February 8, 1960.

Table 1.—Results of field tests for control of codling moth on Bartlett pears. 1959.

Material[a]	Pounds per 100 Gallons	Number of Entries per 700 Fruits	Infested Fruit (%)
Orchard A—Sacramento County[b,c]			
DDT, 50%	2	129	24.1
DDT 50%+ parathion, 25%	2 1	47	6.7
Parathion, 25%	1	54	7.7
Kepone, 50%	2	55	7.8
Trithion, 25%	2	54	7.7
Sevin, 50%	1.5	4	0.5
Ethyl Guthion, 25%	1.5	1	0.1
Ethyl Guthion, 25%	0.75	14	2.0
Orchard B—Solano County[d,e]			
DDT, 50%	2	175	25.0
DDT, 50%+ parathion, 25%	2 1	42	6.0
Parathion, 25%	1	39	5.5
Trithion, 25%	2	35	5.0
Guthion, 25%	1.5	1	0.1
Guthion, 25%	0.75	4	0.5
Guthion[f]	2	10	1.2
Sevin, 50%	1.5	4	0.5
Orchard C—Solano County[g]			
DDT, 50%[d]	2	145	20.7
Guthion, 25%[d]	1.5	0	0.0
Sevin, 50%[d]	1.5	0	0.0
Diazinon, 25%[d]	2	39	5.5
Diazinon, 25%[h]	2	0	0.0
Malathion, 25%[h]	2	2	0.3

[a] All wettable powders unless indicated otherwise.
[b] Application dates—April 9, May 7, June 17.
[c] L.S.D. at .01 = 12.7 for number of entries per 700 fruits.
[d] Application dates—April 7, April 28, June 18.
[e] L.S.D. at 0.1 = 8.31 for number of entries for 700 fruits.
[f] Two pints of emulsion concentrate (1.5 lb./gal.).
[g] L.S.D. at .01 = 14.9 for number of entries per 700 fruit.
[h] Application dates—April 7, 23, May 7, 21, June 4, 18, July 3.

the first two sprays timed to bait pan catches, and subsequent treatments at 3-week intervals. DDT had been used in this orchard since 1946.

The results of the 1959 field plots are summarized in table 1. The poor control obtained in the DDT plot, and the fact that no significant difference was obtained between a combination of DDT-parathion and parathion alone points to a resistance problem in this orchard. It should also be noted that the grower treated the surrounding orchard with Guthion in 1959, which reduced the overall codling moth population. Kepone and Trithion did not provide satisfactory control. In the case of Trithion, a wettable powder formulation was used in an attempt to overcome phytotoxic effects which have been observed with other formulations. The control with wettable powder was considerably poorer than has been obtained in previous seasons using a flowable formulation. Ethyl Guthion and Sevin provided excellent control in this orchard. There was a significant difference between Ethyl Guthion at a dosage of 1.5 pounds and at 0.75 pound. Most of the entries found in the lower dosage were very young worms, which may indicate a lack of residual action.

Observations on other pests showed that Ethyl Guthion held European red mite (*Panonychus ulmi* (Koch)) in check through the harvest season whereas all the other plots required the addition of a specific acaricide to prevent foliage injury by the mites. At harvest it was also evident that mealybugs and aphids were present in economic numbers on the plots treated with DDT, Kepone, and Trithion.

Orchard B reported 75% wormy fruit at the close of the 1958 season. The orchard had received four applications of DDT, timed to bait pan catches. DDT had been used for codling moth control since 1946. The field trials in orchard B are summarized in table 1.

The results were very similar to those obtained in orchard A. A resistance problem was strongly indicated by the poor control with DDT and the lack of a significant difference between DDT-parathion and parathion alone. Here again, the grower treated his surrounding orchard with Guthion. Trithion failed to provide satisfactory control, and both Guthion and Sevin gave excellent results. In this trial, there was no difference between the high and low dosage of Guthion, but there was a significant increase in wormy fruit with the emulsion concentrate formulation as compared with the wettable powder at an equivalent dosage of active ingredient. There was considerable foaming in the spray tank when the emulsion concentrate was used so excess wetting and subsequent run off may explain the results.

The European red mite was also present in these plots, and Guthion was the only compound that did not require the addition of a specific acaricide prior to harvest. Wettable Trithion failed to control mites, but an application of one-half pint Trithion flowable per 100 gallons resulted in satisfactory control. This is additional evidence that the poor control obtained against codling moth with wettable Trithion may be owing to a formulation problem.

Orchard C reported an infestation of codling moth in excess of 50% during 1958. As was the case with the other orchards, DDT was used in a well-timed schedule based upon previous practice in the area. The compound had been the standard control for codling moth since 1946.

The experimental plot results from orchard C are summarized in table 1.

The grower treated his orchard with Guthion, and in spite of the reduced codling moth population the DDT sprayed trees showed a high percentage infested fruit. A resistance problem was also indicated in this orchard. Diazinon did not provide adequate control when used at normal intervals determined by bait pans, while Guthion and Sevin provided excellent control in this schedule. When Diazinon was used at 2-week intervals, control was satisfactory as was the case in the plot which received malathion. The data indicate that Diazinon does not have sufficient residual action to provide control if treatments are spaced a month or more apart.

These results indicate that the problem of DDT resistance by the codling moth is of increasing importance. At the end of the 1959 season, additional reports were received of poor control with DDT even though a number of growers had shifted to substitute compounds. It will now probably be very difficult to determine the extent of resistance to DDT as it is the tendency for growers to employ other materials when a problem is suspected.

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Insecticidal Control of San Jose Scale on Stone Fruits[1]

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ABSTRACT

The following materials applied in the delayed dormant stage gave the best control of San Jose scale (*Aspidiotus perniciosus* Comst.): Volck Supreme oil used alone at 2 gallons or at 1 gallon in combination with Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate) at 1 pint, parathion at 1 pound, malathion at 2 pounds, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) at 2 pounds. Ethion-dormant oil combination at 2 gallons gave good scale control.

Sevin® (1-naphthyl methylcarbamate) used alone has not given satisfactory control, but combined with 1 gallon of Volck Supreme oil has given good control of scale.

Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate)-oil or dimethoate gave partial control, but was not as effective as the other combinations used. Volck Supreme oil at 1 gallon, used alone, did not give satisfactory scale control.

Field studies from 1957 to 1959 have shown that dormant-type oils were more phytotoxic to stone fruit trees than the Volck Supreme oil. However, the Volck Supreme oil was used at a lower dosage with better scale control. With heavy scale infestation, 1½ gallons of Volck Supreme oil is not adequate for good control. All dosages are expressed in amounts per 100 gallons of water and all concentrations of materials are all in terms of the formulations listed in the tables.

Since the San Jose scale (*Aspidiotus perniciosus* Comst.) is a constant threat to stone fruit trees, and in order to find a substitute for parathion, a testing program was conducted during 1957, 1958 and 1959 to evaluate delayed dormant sprays containing the new Volck Supreme oil combined with various organophosphorous compounds for the control of this insect and for phytotoxicity to the trees.

Since the advent of parathion as a delayed dormant or prebloom spray for scale control, this material has in the majority of cases replaced the old dormant spray of oil-lime-sulfur on stone fruits. During the past few years, parathion applied in the delayed dormant stage on stone fruits has not given the excellent control which it did in the earlier years. When applied in late June against the crawler stage, it still gives excellent control.

Volck Supreme oil can be used at a lower dosage and has a greater ease of handling than dormant-type oil. Preliminary trials prior to 1957 showed outstanding control of this scale when 1 gallon of dormant-type oil was added to 1 pound of 25% parathion.

The characteristics of the oils used are as follows:

Phostex® (for chemical definitions of proprietary materials see paragraph preceding "References Cited") 8% and ethion 2.2% were dissolved in a dormant spray oil having a viscosity of 115 (SSU 100° F.), a distillation specification of 15 to 25% off at 636° F., and a UR minimum of 80%. The Volck Supreme has the following characteristics: unsulfonated residue—93%; API gravity—30.6; viscosity 100° F., SSU—142.

Concentrations of materials given in this paper are all in terms of the formulations listed in the tables.

Experimental control work was conducted from 1957–59 in orchards having uniformly heavy populations of scale.

TESTS IN 1957.—Single-tree plots were replicated three times and randomized throughout the orchard. Sprays were applied April 4 to old cherry trees, using a conventional high-pressure srpay machine at 400 p.s.i. Parathion and oil and lime-sulfur were used as the standard treatments.

Treatments were evaluated by counts made by collecting 10 infested twigs at random from each tree and examining them under a dissecting microscope. Fifteen hundred scales per plot were examined in 1957 and 2,000 in 1958–59. Each scale was lifted up with a teasing needle to determine whether it was alive or dead. The percentage of scale control was determined from a pretreatment and one posttreatment scale count. Posttreatment scale counts in 1957 were made 42 days after the spray application.

In a second experiment, Volck Supreme oil was used alone to determine its effectiveness against San Jose scale on prunes. Half of a 1-acre orchard was treated with oil at 1 gallon and the other half with oil at 2 gallons per 100 gallons of water. Sprays were applied and counts were made in the same manner as described above. The results of the 1957 spray experiments are presented in table 1.

TESTS IN 1958.—Twelve materials and combinations of materials were tested during 1958 as delayed dormant sprays for the control of San Jose scale. Sprays were ap-

[1] Scientific Paper No. 1965, Washington Agricultural Experiment Stations, Pullman, Washington. Project No. 1090. These investigations were partially supported by research grants from American Cyanamid Company, Stauffer Chemical Company, Chemagro Corporation and California-Spray Chemical Corporation. Accepted for publication May 23, 1960.

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Table 1.—Control of San Jose scale on cherries treated April 4 with oil alone and in combination with various insecticides. Wenatchee, Washington, 1957.

Materials per 100 Gallons of Water		Per Cent Dead Scale March 3	Per Cent Dead Scale May 16
Parathion 25%, WP	1 lb.	47.6	97.7
Diazinon 25%, WP	2 lb.		
+		38.5	98.3
Volck Supreme Oil 98%, EM	1 gal.		
Parathion 25%, WP	1 lb.		
+		53.3	99.5
Volck Supreme Oil 98%, EM	1 gal.		
Malathion 25%, WP	2 lb.		
+		45.5	100.0
Volck Supreme Oil 98%, EM	1 gal.		
Malathion 25%, WP	3 lb.		
+		42.4	98.2
Volck Supreme Oil 98%, EM	1 gal.		
Trithion 45%, EM	1 pt.		
+		33.6	99.6
Volck Supreme Oil 98%, EM	1 gal.		
Dormant Oil EM	2 gal.		
+		35.1	99.8
Lime Sulfur EM	3 gal.		
Volck Supreme Oil 98%, EM	1 gal.[a]	20.4	95.8
Volck Supreme Oil 98%, EM	2 gal.[a]	16.0	100.0

[a] Sprayed on prunes.

plied and scale counts were made in the same manner as in the 1957 experiments.

The plots were set up in an orchard of large cherry trees severely and uniformly infested with this insect. Half of the orchard was sprayed with a portable hand gun sprayer and half with air carrier equipment. Because of the large size of the trees and abundance of scale in the tops of the trees, this orchard seemed to offer an ideal plot to test the efficiency of these two types of application.

Treatments were applied March 25 by high pressure spray machine to blocks of four large cherry trees. Because of the closeness and large size of the trees, single-tree plots were not practical. All twig samples were taken from those portions of the trees facing the center of the plot to avoid the effects of spray drift. The twig samples were taken 6 to 14 feet above the ground. One count of scale was made before treatment and one after.

A lime-sulfur and Volck Supreme oil combination was applied both by hand and by air carrier equipment for comparison. Parathion alone, and oil plus lime-sulfur were used as the standard treatments. Plot 12 in table 2 gives the data obtained with the air carrier equipment. The materials used, dosages applied and the results of the scale counts are presented in table 2.

Tests in 1959.—San Jose scale control experiments were conducted in a severely infested prune orchard. Sprays were applied by high pressure spray machine and in the same manner as in the 1957 and 1958 experiments. Twenty treatments were applied on April 7–8 in the delayed dormant stage.

Volck Supreme oil was used alone and in combination with a number of the organophosphorous insecticides. Ethion was already formulated with dormant oil. The sprays were applied to single-tree plots replicated four times and randomized throughout the orchard.

Discussion.—In recent years conditions have been favorable for overwintering scale in north central Washington because of the mild winters. When temperatures reach −20° F. or lower, 50% to 60% of the scale is killed. However, counts of overwintering scale made in 1958 and 1959 showed that a high percentage of the scale will survive during mild winters (tables 2 and 3).

The dormant-type oil-lime-sulfur spray applied in the delayed dormant stage in 1957 caused injury to some trees. The nights were cool during this period and typical oil damage, which consisted of necrotic leaves and shock to the trees, was apparent on some of the trees. During the delayed dormant period in 1958 the weather was moderate and no visible injury was observed. Freezing temperatures in 1959 occurred shortly after the sprays were applied and the application containing 4 gallons of dormant-type oil caused severe injury to prune trees. Volck Supreme oil at 1½ gallons applied to scale-weakened trees also caused oil injury. However, trees which were in better condition did not show this injury, even when 1½ to 2 gallons of Volck Supreme oil were used. The amount of injury seemed to be associated with the amount of scale damage, low fertilizer, or vigor of the trees.

The Volck Supreme oil was less deleterious than the dormant-type oils. This may be owing to the lower dosage of Volck Supreme oil used in comparison with the amount of dormant-type oil used for scale control.

When parathion was first used for the control of scale in

Table 2.—Effect of various insecticides applied in the delayed dormant stage March 25 to sweet cherries for control of San Jose scale. Wenatchee, Washington, 1958.

Materials per 100 Gallons of Water		Per Cent Dead Scale Feb. 25–28	Per Cent Dead Scale May 19–23
Parathion 25%, WP	1 lb.	13.3	98.8
Phostex 8%			
+	2 gal.	13.8	99.9
Petroleum Oil 89%			
Volck Supreme Oil 98%, EM	3 gal.	18.6	96.0
Volck Supreme Oil 98%, EM	2 gal.	14.3	100.0
Volck Supreme Oil 98%, EM	1 gal.		
+		19.0	99.4
Parathion 25%, WP	1 lb.		
Volck Supreme Oil 98%, EM	1 gal.		
+		22.0	99.8
Malathion 25%, WP	2 lb.		
Volck Supreme Oil 98%, EM	1 gal.		
+		16.0	99.7
Malathion 25%, WP	3 lb.		
Volck Supreme Oil 98%, EM	1 gal.		
+		16.6	99.6
Trithion 45%, EM	1 pt.		
Volck Supreme Oil 98%, EM	1 gal.		
+		18.5	99.4
Diazinon 25%, WP	2 lb.		
Ethion 2.2%, EM			
+	2 gal.	21.0	99.5
Oil 95%, EM			
Volck Supreme Oil 98%, EM	2 gal.		
+		22.7	99.9
Lime Sulfur	3 gal.		
Volck Supreme Oil 98%, EM	2 gal.		
+		22.8	100.0
Lime Sulfur	3 gal.[a]		

[a] Application made with air carrier equipment.

the prebloom or delayed dormant stage in Washington, excellent control could be obtained (Lanchester & Dean 1957, O'Neill 1951). However, during the experimental tests in 1957 to 1959 the average control was 97.2%.

RESULTS.—Because of windy weather at the time the delayed dormant sprays were applied in 1957, and the lack of a mid-season parathion spray, a mild scale infestation could be found in the tops of the trees in plots 1 to 5 (table 1).

Sevin® used alone for scale control was not satisfactory, but when combined with 1 gallon of Volck Supreme oil gave good control.

Guthion®-oil, or dimethoate-oil combinations gave partial scale control, but were not as effective as the other combinations tested. Malathion-oil mixtures were fairly effective. Diazinon®-oil gave excellent control of scale in 1958–59, and fair control in 1957. The majority of the organophosphorous insecticides, when combined with oil, gave excellent control of scale.

The combinations of 1 gallon of Volck Supreme oil with the following compounds have all given good control of San Jose scale: Trithion® at 1 pint, parathion at 1 pound or malathion at 2 pounds. Ethion-oil combination at 2 gallons per 100 gallons of water also gave good scale control.

Whenever there was a severe infestation of scale, 1 or 1½ gallons of Volck Supreme oil was not adequate to obtain good control. However, when used at 2 gallons per 100 gallons of water this oil gave good scale control.

Chemical definitions of materials bearing proprietary names referred to in this paper are:

Diazinon (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate)

Guthion (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate)

Phostex (a mixture of bis(dialkyloxyphosphinothioyl) disulfides)

Sevin (1-naphthyl methylcarbamate)

Trithion (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)

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Table 3.—Mortality of San Jose scale on prunes following the application of experimental sprays on April 7–8. Wenatchee, Washington, 1959.

MATERIALS PER 100 GALLONS OF WATER		PER CENT DEAD SCALE FEB. 13–MARCH 13	PER CENT DEAD SCALE JUNE 3–8
Volck Supreme Oil 98%, EM	1.5 gal.	4.1	93.0
Volck Supreme Oil 98%, EM	2 gal.	10.5	89.4
Dormant Oil 80%	4 gal.	5.6	95.2
Dormant Oil 80%	1.5 gal.	3.9	95.2
Parathion 25%, WP	1 lb.	3.5	95.2
Parathion 25%, WP	1.5 lb.	8.2	96.5
Volck Supreme Oil 98%, EM	1 gal.		
+		2.5	99.3
Lime Sulfur	3 gal.		
Dormant Oil 80%, EM	2 gal.		
+		5.3	95.9
Lime Sulfur	3 gal.		
Volck Supreme Oil 98%, EM	1 gal.		
+		10.9	99.2
Trithion 45%, EM	1 pt.		
Sevin 50%, WP	2 lb.	9.7	87.9
Sevin 50%, WP	1 lb.		
+		14.0	99.3
Volck Supreme Oil 98%, EM	1 gal.		
Parathion 25%, WP	1 lb.		
+		8.6	96.2
Volck Supreme Oil 98%, EM	1 gal.		
Parathion 25%, WP	1 lb.		
+		11.6	99.05
Dormant Oil 80%, EM	1 gal.		
Malathion 25%, WP	2 lb.		
+		8.4	96.5
Volck Supreme Oil 98%, EM	1 gal.		
Diazinon 25%, WP	1 lb.		
+		42.7	99.9
Volck Supreme Oil 98%, EM	1 gal.		
Ethion 2.2%			
+	2 gal.	7.4	99.9
Oil 95%			
Dimethoate 46%, EM	1 pt.		
+		15.8	80.5
Volck Supreme Oil 98%, EM	1 gal.		
Guthion 17.5%, EM	1.5 pts.		
+		20.5	92.6
Volck Supreme Oil 98%, EM	1 gal.		
Parathion 25%, WP	1 lb.		
+		31.1	94.5
Plyac (Surfactant)	8 oz.		
Check (untreated)		9.9	0
Volck Supreme Oil 98%, EM	1.5 gal.[a]	19.6	99.6

[a] Application made with Speed Sprayer.

Corn Earworm Oviposition and the Effect of DDT on the Egg Predator Complex in Corn Silk[1]

FLOYD P. HARRISON, *University of Maryland, College Park*

ABSTRACT

During the growing season of 1959, observations were made on five plantings of sweet corn to determine some of the oviposition habits of the moth and to study the effect predators have on the mortality of eggs of the corn earworm (*Heliothis zea* (Boddie)). The plantings were made on May 15, May 25, June 4, June 14 and June 24.

Earworm oviposition was relatively light in the first three plantings. In the last two plantings egg deposition increased considerably. This increase in activity is usually observed to occur late in the corn-growing season. Newly emerged silks were most attractive to the moths but even after silks were 10 days old, moths were observed to oviposit on them.

The main predators responsible for egg mortality were *Orius insidiosus* (Say), *Coleomegilla maculata* (DeGeer) and *Chrysopa oculata* Say. *Geocoris punctipes* (Say), *Nabis ferus* (L.) and syrphid larvae were also observed in the silks but in very limited numbers. The effects of treatment with DDT-oil emulsion sprays on predator populations and egg mortality were also observed. Where spraying reduced the number of predators, mortality of earworm eggs was decreased.

Observations made by the author during a period of 3 years while studying the timing of DDT-oil emulsion sprays for control of corn earworm, *Heliothis zea* (Boddie), indicated that improperly timed applications sometimes caused an increase in earworm injury. This is not the first time such observations have been recorded. Ewing & Ivy (1943) stated, "Timely applications of heavy dosages of calcium arsenate control the bollworm, but when the dosage is too light or applications are not made at the proper time, cotton dusted with calcium arsenate becomes more heavily infested with bollworms than does undusted cotton." They also stated that this might be owing to the effects of insecticides on the predators of *Heliothis*. Among the several authors who have observed the association between *Heliothis* eggs and predacious insects and the effects insecticides have on these predators are Newsom & Smith (1949), Campbell & Hutchings (1952), and van den Bosch *et al.* (1956).

The study discussed herein was initiated to: (1) observe egg-laying activity on corn silks; (2) determine the insect species that prey on eggs of *Heliothis zea* in corn silks; (3) correlate oviposition by *Heliothis zea* with the abundance of silk inhabiting predaceous insects; (4) determine the effect that insecticidal sprays have on populations of predators, and the effect reduced populations of predators have on egg mortality.

METHODS.—In 1959, five plantings, each one-quarter acre in size of Io Chief sweet corn, were made May 15, May 25, June 4, June 14, and June 24. Each planting was divided into two equal plots, each $\frac{1}{8}$ acre in size. One plot was left untreated; the other was sprayed with a DDT-oil emulsion containing 1 pound of DDT and 7 quarts of white mineral oil applied to the silks in 25 gallons of spray per acre. Two applications were made to each treated plot, the first when approximately 50% of the plants were in silk and once 4 days after the first treatment was applied. The sprays were applied with a high clearance sprayer with four 6501 "*Tee Jet*" nozzles per row. Twenty-five ears in each plot, all of which silked on the same day, were tagged for observation. These ears were marked on the third day after the first silk appeared. This was when about 25% of the plants were in silk. Daily, usually early in the morning, records were taken of the eggs deposited during the preceding crepuscular hours and of the predators inhabiting the corn silks. As each egg was deposited and located on the silk, it was tagged using a method similar to that used by Ditman (1931). A small strip of paper bearing a number was glued around the silk strand about $\frac{1}{8}$ inch above the egg. The egg could then easily be found on succeeding examinations. Observations were made on each marked ear for 10 days. After 10 days the silks were dead and the ears were nearing the roasting-ear stage.

In undertaking a study of the predators that attack eggs of the corn earworm, there arose a need for a method by which the effects of the individual species of egg predators could be measured under natural conditions and by which an accurate measure of the relative importance of each predator species could be obtained. In preliminary observations in 1958 viable earworm eggs and also what appeared to be the remains of chorions of earworm eggs could be found in fresh corn silks. A simple experiment was designed to determine the significance of these observations.

Corn silks with freshly deposited earworm eggs were confined in large vials, each with several individuals of one predator species. There was also a vial of eggs which contained no predators. The predator species observed here were: the anthocorid bug, *Orius insidiosus* (Say); the adults and larvae of the coccinellid beetle, *Coleomegilla maculata* (DeGeer), and larvae of the golden-eye lacewing, *Chrysopa oculata* Say. Within a relatively short time each predator fed on the eggs within the vials. The remains of the eggs could be readily-correlated with the predator species when observed through a binocular microscope or hand lens. In other words, each species damaged the eggs in a characteristic way. Also normally hatched eggs could be classified as such and separated from the eggs that had been attacked. The same phenomena were observed in the field. Figure 1 is a diagrammatic drawing of the remains of earworm eggs after normal hatching (A), after having been attacked by *Orius* (B), and by *Coleomegilla* (C).

Quaintance & Brues (1905) described the hatching of

[1] Scientific Article No. A855, Contribution No. 3153 of the Maryland Agricultural Experiment Station, Department of Entomology. Accepted for publication May 24, 1960.

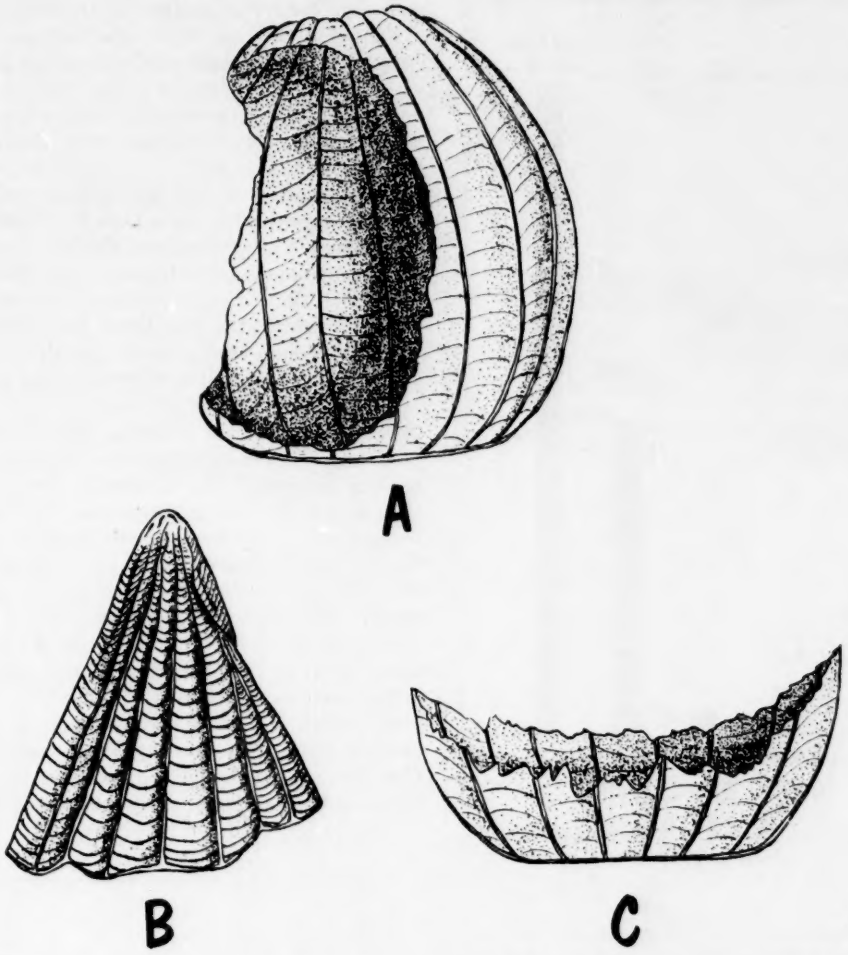

FIG. 1.—Diagrammatic drawings of corn earworm eggs after hatching (A), after having been attacked by *Orius insidiosus* (B), and by *Coleomegilla maculata* (C).

earworm eggs as follows: "The exit hole is usually large, with very ragged edges, initiated on one side of the egg . . ." As illustrated in figure 1, A, this was exactly the case in these observations. There was some variation in the size of the exit hole. Quaintance & Brues (1905) also described the eating of the chorion by newly hatched larvae. This was observed repeatedly throughout this study, and the exit holes varied in size according to the amount of chorion consumed by the young larvae.

Invariably, eggs attacked by *Orius* attained a teepee shape (fig. 1, B). This was the least difficult of the egg shells to identify because of the consistent and uniform shape.

Both adults and larvae of *Coleomegilla* attacked earworm eggs by chewing and leaving only fragments of the chorion as shown in figure 1, C. The appearance of eggs attacked by this species varied somewhat, but only in a few instances was there any question as to whether an egg had been eaten by this species. Often the chorion would be chewed down to the base of the egg, but the characteristic of *Coleomegilla* chewing remained evident.

Larvae of *Chrysopa* feed with a sucking action utilizing a tube formed by the elongated mandibles and maxillae (R. C. Smith 1922). The sucking action causes a general collapse of the egg. This feeding damage was the most difficult to recognize. Attacked eggs took several forms, therefore, a generalized illustration of this is not given. The lack of a pattern as to the collapse of the chorion usually helped identify the predatory work of *Chrysopa*. Numerically this insect was not of great importance and any error therefore would not be large. There were other predatory insects, as *Nabis ferus* (L.) and *Geocoris punctipes* (Say) and larvae of the syrphid flies that frequent corn silks, but because of their small numbers sufficient information is not available to describe the damage they do to *Heliothis* eggs.

RESULTS.—Figure 2 shows the egg-laying activity observed in these plantings. The number of eggs deposited per silk in the first planting was small, slightly less than one per silk. This small number dropped even lower in the next two plantings. Only on the plantings of June 14 and June 24 did activity increase to a level above one egg per

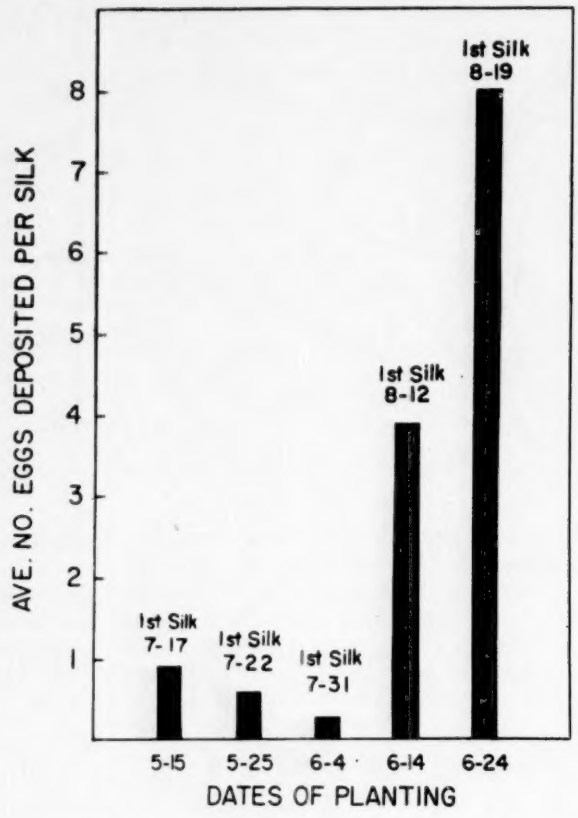

FIG. 2.—Egg-laying activity of corn earworm in five plantings of 1959.

silk. This midseason drop and late-season increase was explained by Dicke (1939) as being caused by a population dilution. Late-season increase has also been observed by the author (Harrison & Ditman 1958). Figure 3 gives the egg-laying activity relative to the age of the ear. The fresh new silk was most attractive to the moth. Egg-laying activity fell off until the last observations were made on the 10-day-old silks; however, after 10 days, though the silks were dead, moths found them somewhat attractive.

Several times during the course of these observations, collections of the predators were made and the species determined. These species were *Orius insidiosus*, adults and larvae of *Coleomegilla maculata*, *Chrysopa oculata*, *Nabis ferus* and *Geocoris punctipes*. It is possible that other species of these genera were present in small numbers.

Figure 4 gives the relative abundance of the predators found in the corn silks and their seasonal occurrence. *Orius* was found in greater numbers than any other predator. The next most numerous were larvae of *Coleomegilla*. *Chrysopa*, *Nabis* and *Geocoris* occurred in smaller numbers. Figure 4 also gives the seasonal occurrence of the predators. When this is compared with figure 2 it is apparent that as oviposition by *Heliothis* reached its highest peak in the last planting, *Orius* declined in numbers. In the period of least earworm activity, *Orius* was more numerous in the corn silks than at any other time.

Figures 5 through 9 give the comparative results of daily population counts of the observed predator species in untreated and treated plots. Spraying reduced the number of predaceous insects in the silks, but *Orius*, the most numerous, was not completely eliminated in any of the sprayed plantings.

An evaluation of the egg mortality caused by each predator species is shown in table 1. *Orius* destroyed more eggs than any other predator. Table 1 also shows the predation in treated and untreated plots. Table 2 shows the activity of the various predaceous insects in the treated plots before and after treatment. Both tables indicate the effects of reduction of predator populations on egg survival. It is apparent that sprays reduce egg mortality.

DISCUSSION.—There was not a close correlation between the number of *Heliothis* eggs and the number of predators. This is especially evident with *Orius*. As oviposition increased, the number of *Orius* inhabiting the silks decreased. This suggests that there was a more important source of food for *Orius* than earworm eggs. Barber (1936) observed that the presence of *Orius* was more or less coincidental with the supply of eggs, but that the availability of eggs is not all together responsible for the rise and fall of *Orius* populations. Ewing & Ivy (1943) noted that the abundance of cotton aphids permitted larger numbers of bollworms to hatch than when aphids were absent. This also suggests that *Orius* is more dependent upon the cotton aphid for food than it is upon *Heliothis* eggs. Although data are not presented here on the abundance of corn leaf aphids, *Rhopalosiphum maidis*

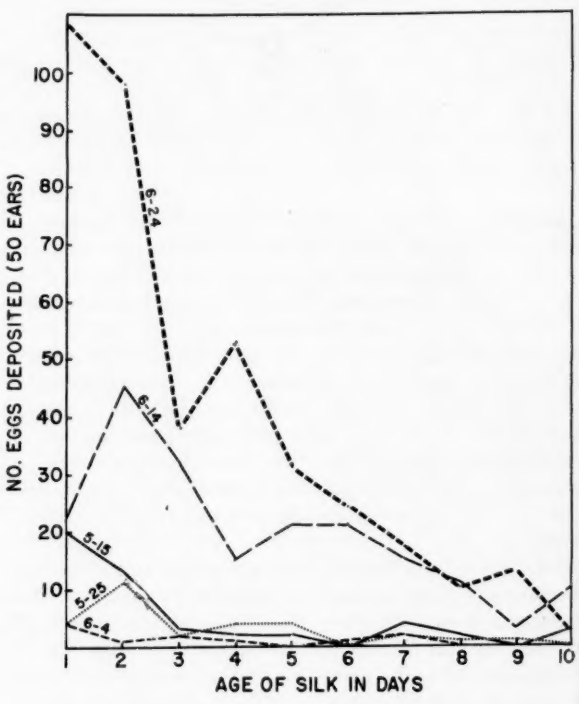

FIG. 3.—Relation between the age of the corn silk and attractiveness to the earworm adult for oviposition in five plantings in 1959.

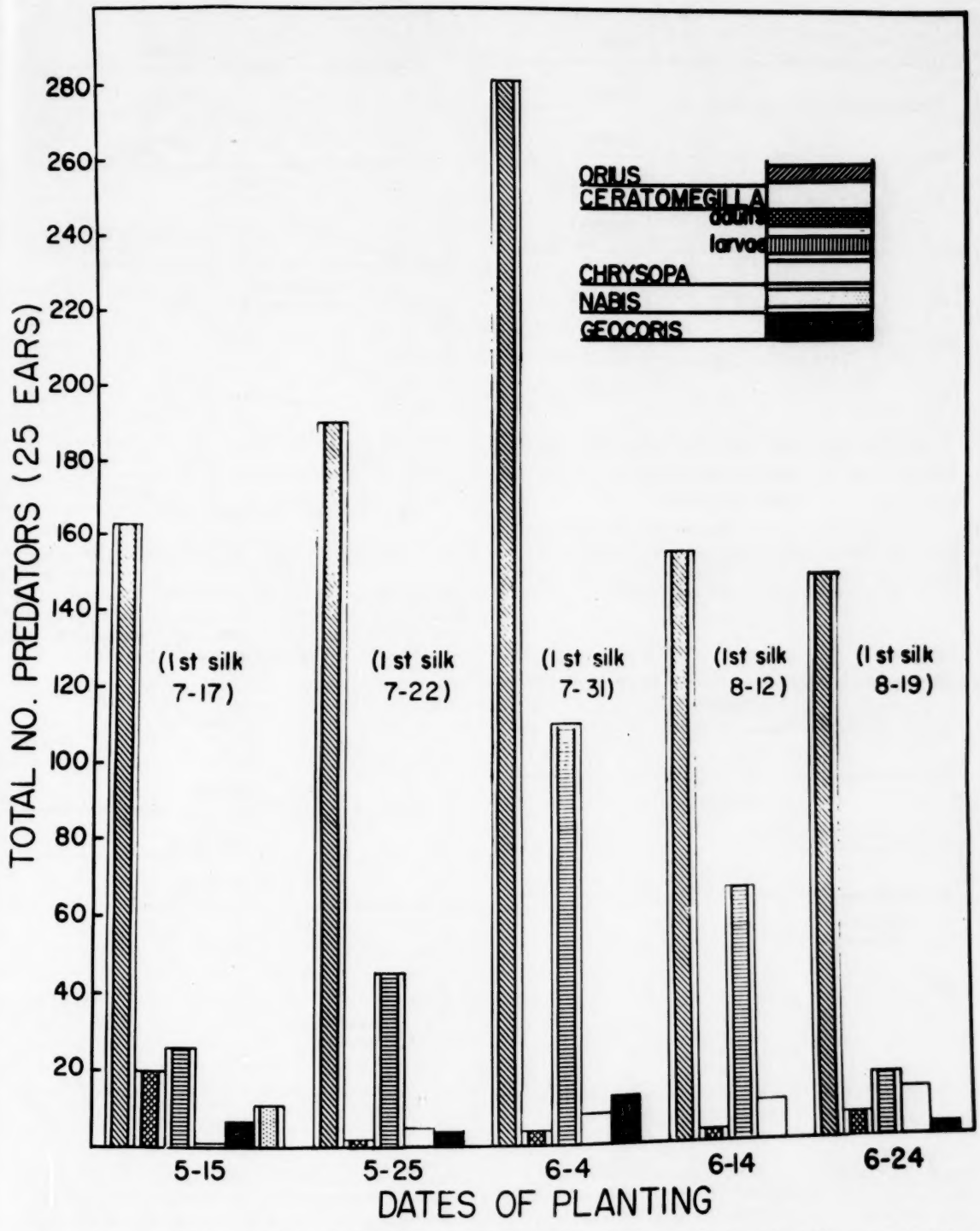

FIG. 4.—Relative abundance of the predators inhabiting corn silk in five plantings made throughout the season of 1959.

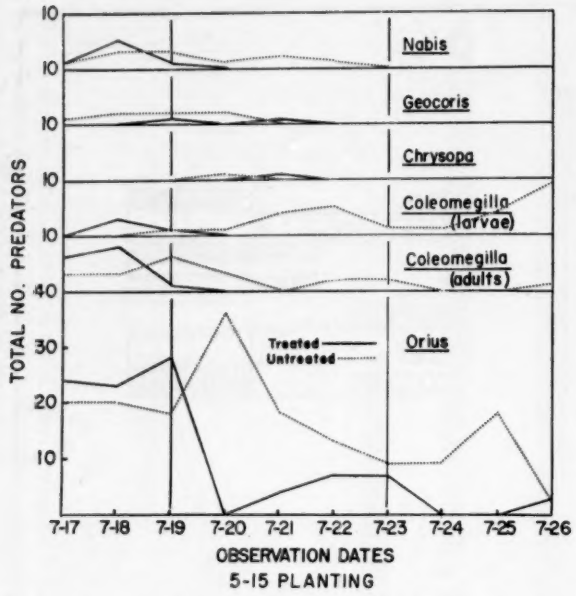

FIG. 5.—A comparison of the number of predators found in treated and untreated plots of sweet corn (based on 25 ears from each) planted May 15, 1959. Inside vertical lines represent the dates treatments were applied.

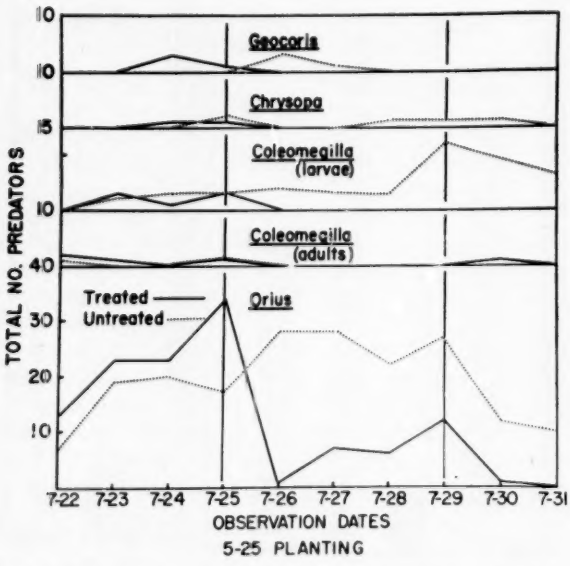

FIG. 6.—A comparison of the number of predators found in treated and untreated plots of sweet corn (based on 25 ears from each) planted on May 25, 1959. Inside vertical lines represent the dates treatments were applied.

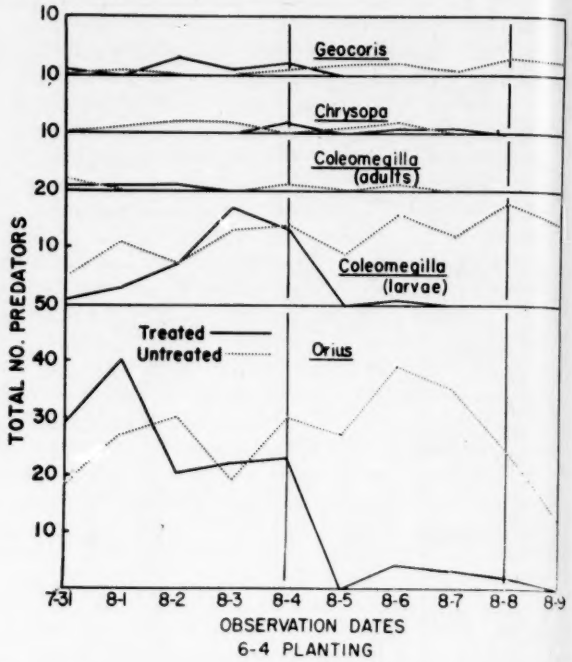

FIG. 7.—A comparison of the number of predators found in treated and untreated plots of sweet corn (based on 25 ears from each) planted on June 4, 1959. Inside vertical lines represent the dates treatments were applied.

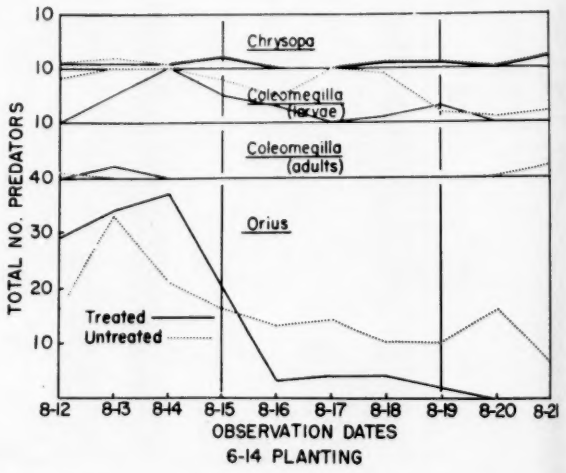

FIG. 8.—A comparison of the number of predators found in treated and untreated plots of sweet corn (based on 25 ears from each) planted on June 14, 1959. Inside vertical lines represent the dates treatments were applied.

Table 1.—The fate of *Heliothis* eggs deposited on corn silks in five plantings in 1959.

Category	No. Eggs[a]	Per Cent				Preyed Upon by			Total Predation (%)	Eggs on Silk at Last Observation (%)
		Hatched	Infertile	Unaccounted for	Lost Mechanically	*Orius*	*Coleomegilla*[c]	*Chrysopa*		
					May 15 Planting					
Untreated	36	33.3	0	27.8	11.1	22.2	0	5.6	27.8	0
Treated[b]	10	30.0	20.0	40.0	0	0	0	10.0	10.0	0
					May 25 Planting					
Untreated	12	58.3	8.3	8.3	0	0	25.0	0	25.0	0
Treated	18	16.6	22.2	5.6	33.3	5.6	0	16.7	22.3	0
					June 4 Planting					
Untreated	9	22.2	0	22.2	11.1	11.1	11.1	0	22.2	22.2
Treated	9	33.3	0	0	11.1	0	11.1	22.2	33.3	22.2
					June 14 Planting					
Untreated	101	27.7	2.0	13.9	10.1	19.8	9.9	3.9	36.3	11.9
Treated	87	48.3	2.3	10.3	8.0	12.6	0	3.4	16.0	14.9
					June 24 Planting					
Untreated	220	43.6	6.8	15.9	6.8	15.0	5.4	3.6	24.0	2.7
Treated	183	45.4	5.4	15.8	10.9	8.2	0	6.0	14.2	8.2
					Weighted means for all five plantings					
Untreated	75.6	38.4	4.8	16.4	8.2	16.4	6.9	3.7	26.9	5.3
Treated	61.4	43.6	5.8	14.0	10.7	8.8	.3	6.2	14.9	9.8

[a] Total deposited on 25 ears in each plot.
[b] Treated plots were sprayed with 1 pound of DDT—7 quarts of white mineral oil in 25 gallons of water per acre when approximately 50% of the plants were in silk and again 4 days later.
[c] Larvae and adults.

Table 2.—A comparison of predator activity before and after treatment with DDT-oil emulsion in the treated plots in five plantings of sweet corn in 1959.

Planting Date	Before Treatment					After Treatment				
	No. Eggs Deposited in 25 Ears	Per Cent Eggs Attacked by			Destroyed by Predators (%)	No. Eggs Deposited in 25 Ears	Per Cent Eggs Attacked by			Destroyed by Predators (%)
		Orius	*Coleomegilla*[a]	*Chrysopa*			*Orius*	*Coleomegilla*[a]	*Chrysopa*	
May 15	5	0	0	0	0	5	0	0	0	0
May 25	16	6.3	0	18.8	25.1	2	0	0	0	0
June 4	4	0	25.0	0	25.0	5	0	0	0	0
June 14	47	21	0	4.3	25.3	40	2.1	0	2.1	4.2
June 24	133	11.3	0	7.5	18.8	50	0	0	2.0	2.0

[a] Larvae and adults.

(Fitch), it was noted during this work that there were considerably fewer aphids in the June 14 and June 24 plantings than in the three earlier plantings. Perhaps aphids on the corn silks, husks and other places about the corn plant were more important as a source of food for *Orius* than were corn earworm eggs.

Tables 1 and 2 show how a reduction in the number of predators may influence egg survival. A comparison of the predator activity before and after treatment may indicate that applications of DDT sprays made earlier than those reported here may adversely affect the results of such sprays.

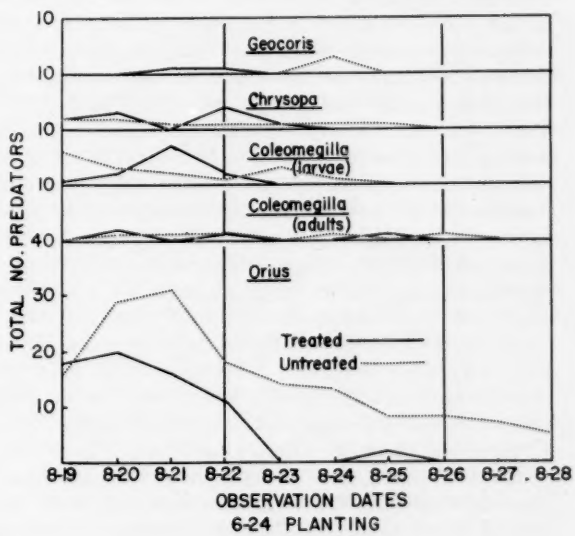

FIG. 9.—A comparison of the number of predators found in treated and untreated plots of sweet corn (based on 25 ears from each) planted on June 24, 1959. Inside vertical lines represent the dates treatments were applied.

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Establishment of Imported Parasites of the Spotted Alfalfa Aphid In Arizona[1]

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ABSTRACT

About 235,000 pupae and adults of *Aphelinus semiflavus* Howard, *Praon palitans* Muesebeck, and *Trioxys utilis* Muesebeck were shipped to Arizona between November 1955 and July 1957 for release in alfalfa fields infested with the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)). Releases were made at several sites, the number at a single site ranging from 250 to more than 56,000. *A. semiflavus* has been recovered in small numbers at several locations in central Arizona, the last recovery being in April 1959 about 100 miles north of the nearest release site. *P. palitans* has been recovered several times in central and southwestern Arizona. In 1959 it was collected from five locations in the south-central and two in the north-central part of the State, but was of no consequence in control of the spotted alfalfa aphid. *T. utilis* is generally established in the alfalfa-growing districts of southern Arizona and is a substantial factor in controlling the aphid.

Three imported hymenopterous parasites of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) have been discussed by Van den Bosch (1956, 1957). The parasites were *Aphelinus semiflavus* Howard, *Praon palitans* Muesebeck, and *Trioxys utilis* Muesebeck, the last two recently described by Muesebeck (1956). Studies on their biologies were reported by Schlinger & Hall (1959). Importation, mass production, establishment, and role of the parasites in control of this aphid in southern California were discussed by Van den Bosch *et al.* (1959a, 1959b). This paper summarizes the progress in liberation and establishment of the parasites in Arizona.

SHIPMENTS.—About 235,000 adults and pupae of the parasites were mass-produced at Albany and Riverside, California, and Moorestown, New Jersey, and shipped for release in southern Arizona. The mass propagation was handled in cooperation with the University of California Department of Biological Control. The shipments were made by air to Tempe or Yuma during the period November 1955 to July 1957. The distribution of the parasites is shown in table 1. About 78% were released in the south-central area (near Tempe and Litchfield Park in Maricopa County, and Florence in Pinal County) and the rest in the southwestern area (near Yuma in Yuma County). Most of the alfalfa-growing valleys in these areas are at an elevation of 150 to 1,500 feet and the climate is very hot and dry. Each species was included in the releases made in each area. In addition, 300 pupae of *T. utilis* were collected from alfalfa near Tempe and released in alfalfa near Tucson early in July 1958.

Observations on samples of parasite pupae in or under the mummified skins of spotted alfalfa aphids on alfalfa leaves or stems indicated that very large percentages survived shipment and emerged as adults. Counts of dead adults at the time of release were made of shipments to Tempe involving 48,775 adults. The average survival for each species was near 90% (table 2).

METHODS OF RELEASE.—The parasites were usually liberated in alfalfa fields within 24 to 48 hours after shipping. Pupae were within or attached to aphid mummies on alfalfa stems or leaves which were packed in mailing tubes or stout cardboard boxes. Adults were packed in pint or half-pint ice-cream cartons loosely filled with excelsior. Pupae on stems were released by clipping the stems to aphid-infested plants, and those on entire plants by placing small handfuls at short intervals along an irrigation border ridge in an aphid-infested field. Adults were released by removing the lids and placing the cartons

[1] Accepted for publication May 31, 1960.

[2] In cooperation with Arizona and California Agricultural Experiment Stations, D. M. Tuttle, Arizona Agricultural Experiment Station, and V. D. Roth, formerly with the Entomology Research Division, Agric. Res. Serv., U.S.D.A., released parasites and checked on their distribution and establishment in the southwestern part of Yuma County. J. K. Holloway, Entomology Research Division, Agric. Res. Serv., U.S.D.A. gave much helpful advice on release methods.

Table 1.—Numbers of spotted alfalfa aphid parasites received for liberation in Arizona.

SPECIES	SOUTH-CENTRAL AREA	SOUTHWESTERN AREA
Aphelinus semiflavus	86,000	19,500
Praon palitans	82,000	29,000
Trioxys utilis	16,000	2,500
Total	184,000	51,000

on the ground or in the crown of the plants. The adults would leave the cartons and walk or fly to the alfalfa plants readily. Minimum, maximum, and average numbers per carton were 945, 2,025, and 1,371 for *A. semiflavus;* 300, 500, and 377 for *P. palitans,* and 170, 550, and 342 for *T. utilis.*

Most of the releases were made in fields that had received no insecticide application for 6 weeks or longer and in which no early treatment was anticipated. Some farmers cooperated by leaving portions of fields untreated. There was usually one release site per field, and it comprised only a small part of the field, generally in or near a corner. In 1955 only 250 to 500 pupae of a single species were liberated at a site. Larger and more concentrated releases were made in 1956 and the first half of 1957. Sometimes 12,000 to 26,000 adults or pupae of two or three species were liberated at one time and other releases made at the same site on other dates. Some of the highest totals at individual sites were 56,695 in one field near Litchfield Park, 37,584 in another near Tempe, and 27,150 in a third near Yuma. Nearly all the releases were made in Chilean or African alfalfa, varieties that are very susceptible to the spotted alfalfa aphid. No attempt was made to select highly infested fields, but most of those used had one or more aphids per leaf. The range was 0.1 to 38 aphids per leaf and the average 6.7.

About 10 to 15 days after the parasites were liberated a search was made at the release site and in the surrounding alfalfa for parasitized mummies of the spotted alfalfa aphid. Thereafter, periodic inspections were made to check on the progress and spread of the parasites.

The percentage of parasitization was estimated from counts of living aphids and dead aphid mummies on alfalfa leaves. Where aphid populations were moderate to high, the counts were usually made on 30 leaves, one each near the top, middle, and bottom portions of plants at 10 points in each field. Where the aphid population was low, more leaves were included in the counts. In some surveys an attempt was made to examine 100 to 500 aphids in each field sampled, the number depending on the aphid population and time available.

RECOVERIES.—*Aphelinus semiflavus.*—From 1,000 to 36,445 of this species were released at each of eight sites. Between mid-April 1956 and early October 1957 recoveries were made in spotted alfalfa aphid mummies at or near five of the sites in the south-central area. They were made in various months from February through November, except June and August. Parasitization was light, the highest observed being 1.4% of a sample of 500 aphids counted October 2, 1957, in plots at Tempe in which 3,000 pupae had been released between March 14 and July 11 of that year and alfalfa cutting dates had been staggered to maintain some tall green growth. In June a few parasitized aphids were observed up to one-half mile from the nearest release site.

Table 2.—Survival of adult parasites of the spotted alfalfa aphid following shipments to the release area near Tempe, Ariz., 1956–1957.

SPECIES	NO. OF SHIPMENTS	NO. OF PARASITES		SURVIVAL (%)
		Shipped	Survived	
Aphelinus semiflavus	7	15,840	14,331	90.0
Praon palitans	10	17,750	15,711	88.5
Trioxys utilis	24	15,185	13,768	90.6
Total and average		48,775	43,810	89.8

No parasites of this species have been observed in southern Arizona since the fall of 1957. A few parasitized aphids were taken near Camp Verde early in May 1959. The location was in north-central Arizona at an elevation between 3,000 and 3,500 feet and considerably cooler than the lower valleys in south-central and southwestern Arizona. This species does not appear to be well adapted to the hot desert valleys of Arizona. Similar observations were reported in California by Van den Bosch *et al.* (1959a).

Praon palitans.—This species was released at 13 sites and recovered at 8 of them between October 1956 and September 1957. The number released at one site ranged from 400 to 26,200. Recoveries were made at or near some of the release sites in both the south-central and southwestern areas and in all months from February to July and from September to November. In 1957 parasitization was low, the highest observed being 1.1% on April 9 in field plots at Tempe in which 2,200 adults were released February 19 of that year and lots of 6,000 pupae each on March 14 and 26. On July 15 a few parasitized aphids were found about $2\frac{3}{4}$ miles east of the nearest release site near Tempe. In 1958 the parasite was recovered at only one location. This was near Gilbert late in December in a field about 5 miles north and 4 miles east of the nearest release site. Recoveries were more numerous and widespread in 1959. Between January 28 and May 4 small numbers of parasitized aphids were observed in one field near Gilbert, one near Mesa, and one near Perryville, and in two fields near Tempe, all in Maricopa County at elevations between 800 and 1,200 feet, also in one field near Camp Verde and one near Bridgeport, both in Yavapai County at elevations between 3,000 and 3,500 feet. The last two locations are about 100 miles north of the nearest release site.

It appears that *P. palitans* has established itself in south-central Arizona and spread considerably northward but has not increased sufficiently to be of economic consequence in control of the spotted alfalfa aphid.

Trioxys utilis.—Releases of 384 to 4,725 of these parasites were made between November 9, 1955, and June 7, 1957, at 13 sites in the south-central and southwestern areas. In 1957, in the months of March, April, May, June, September, and October, parasites were recovered at three of the sites, one each in Maricopa, Pinal, and Yuma Counties, widely scattered over the release areas. Parasitized aphids were found most frequently and

in greatest numbers in a ¼-acre plot at Tempe in which eight releases totaling 4,725 adults were made between February 19 and May 29. Aphid counts in this plot on April 9 and October 2 indicated 3.28% and 12.2% parasitization, respectively. Between July and September, *T. utilis* spread over about 100 square miles in alfalfa-growing lands in the Yuma and South Gila valleys of southwestern Arizona, but was of little economic importance for spotted alfalfa aphid control because of the low rate of parasitization. Observations from late October to early December in the south-central area, including Maricopa and Pinal Counties, disclosed no parasites and very low aphid populations. However, on December 12 one aphid parasitized by *T. utilis* was found in a field 2¼ miles south of Tempe and 3 miles from the nearest release site.

In 1958 *T. utilis* spread rapidly and widely. By March it was present generally in a 3- by 14-mile area immediately southeast, south, and southwest of Tempe. Parasitization reached 5% to 10% in some fields but was usually lower. Surveys in April, May, and June revealed parasitized aphids over a wider area that included most of the Salt River, Buckeye, and Arlington Valleys in Maricopa County and the Florence and Casa Grande districts in Pinal County, and extended from Arlington to Florence, a distance of about 115 miles. Parasitization usually ranged from less than 1% to 20%, but in a few fields reached 40% to 90%. Evidently, this parasite was of some economic value in reducing populations of the spotted alfalfa aphid in south-central Arizona during the spring of 1958. Later surveys, mostly in October, November, and December, showed that it was widely distributed in aphid-infested alfalfa in Maricopa County; the Yuma, South Gila, Roll, and Aztec districts of southern Yuma County; the Florence, Coolidge, Randolph, Picacho, Casa Grande, and Maricopa districts of Pinal County; near Tucson in Pima County; near San Carlos in Gila County; and the Geronimo, Cork, Safford, and Solomon districts along the Upper Gila Valley in Graham County. Parasitization ranged up to 90% in some fields with very low aphid populations, but in most fields was below 10%. The average was 14.6% in 31 fields sampled during November and December. By the end of 1958 *T. utilis* was apparently well established in the principal alfalfa-growing areas across the southern third of Arizona.

Surveys were continued in 1959 to observe the spread and abundance of this parasite in Arizona. Aphid counts in several alfalfa fields in the north-central and east-central areas during the spring and summer disclosed the parasite early in April in small numbers in one field near Camp Verde, Yavapai County. Surveys of 15 to 22 fields in the south-central area, comprising Maricopa and Pinal Counties, showed that the average parasitization was 39% on February 16 to 19, declined rapidly early in the spring, and remained low for the rest of the year, being only 0.16% on October 16 to 29.

In 1960 parasitization remained very low through January and the early part of February, but by February 15 and 29, and March 11 it had increased to 4.9%, 7.4%, and 13.6%, respectively, in fields sampled in Maricopa County.

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COMMERCIAL AND EXPERIMENTAL ORGANIC INSECTICIDES

By Eugene E. Kenaga

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The Blackheaded Fireworm of Cranberry—A Pest of the Evergreen Huckleberry in Western Washington[1]

E. P. Breakey[2]

Abstract

Moths identified as adults of the blackheaded fireworm of cranberry, *Rhopobota naevana* (Hbn.), were obtained from larvae that were very destructive to the "evergreen huckleberry," *Vaccinium ovatum*, Pursh., on the Olympic Peninsula in Washington in 1958 and 1959. These larvae are known locally as "tipworms" from their habit of feeding on the terminal growth of the young shoots of the host plant. The "evergreen huckleberry" is a new host for the blackheaded fireworm in America. Previously it was known only from cranberry. Two parasitic insects were reared from the "tipworms," an ichneumonid wasp, *Horogenes* sp., and a tachinid fly, *Eulasonia* sp. Both are new records. The parasitic wasps killed the "tipworms" in the last larval stage and destroyed about 10% of them. The synonomy of the blackheaded fireworm is questioned and reasons for this uncertainty are given.

The "evergreen huckleberry"[3] is indigenous to western Washington. Large stands are found on the Olympic Peninsula, principally in Kitsap, Mason, Pierce and Thurston Counties. It is a clustered, well-branched rigid shrub, 0.5 to 3 meters in height, with pubescent, usually reddish branchlets. The leaves are evergreen, very numerous, narrowly ovate, 2 to 3.5 centimeters long, serrate, acute, glossy green above and dull beneath. "Evergreen huckleberry" brush is highly prized as a decorative green. Florists use the glossy, dark green sprays of finely cut leaves as filler and background foliage. The State Department of Natural Resources estimates the "evergreen huckleberry" brush market has a value of at least $1,000,000.00 annually in western Washington (O'Donnell 1959).

Fruit of the "evergreen huckleberry" is also a valuable crop. The berries are globose, 3 to 5 millimeters in diameter (some may be larger), glossy black or glaucous, and are harvested in October. In 1948, the M. E. Mercer Company packed and shipped more than 350 tons of wild "huckleberries" from the Puget Sound area (Shaw 1952). Recently (1959), Mrs. Mercer stated they had packed and shipped as many as 500 tons in a year and that 1,000 tons would be a fair estimate for the total production of the Puget Sound area in some years.

Historical Resumé.—Several insects are known to feed on the evergreen huckleberry. One of these, a lepidopterous larva that is known locally as the "tipworm," is the subject of this article. On June 11, 1958, some of these "tipworms" were brought to the Western Washington Experiment Station. They had been found in Pierce County feeding on the new growth of the "evergreen huckleberry" on May 15, 1958, and were thought to be larvae of the holly bud moth, *Rhopobota naevana ilicifoliana* (Kearfott) which had spread to the "evergreen huckleberry."

The area around Vaughan, Lakebay and Keycenter in Pierce County was visited as soon as possible but it was too late in the year to get living larvae. A search for pupae in the injured tips was also fruitless. The damage was extensive and serious. It was estimated that 30% to 40% of the tips on the brush had been destroyed, halting its growth and making it unfit for florists' greens.

Chemical Control.—No "tipworms" were found on April 9, 1959. Those acquainted with the situation insisted they should be present at about this time. Since the insect *apparently* belonged to the family Tortricidae, it was decided to apply the insecticide TDE as soon as new growth started, about the middle of April. One of the areas that had been cleared up and made accessable was selected for the tests. The spray was prepared to contain 50% TDE at the rate of 2 pounds of the wettable spray powder per 100 gallons. Application was made with a knapsack sprayer. Each plot was carefully marked. All plots were randomized and replicated. Because this test was preliminary and exploratory, only one concentration of TDE was used, *i.e.*, the concentration that was known to be effective against other leaftiers, particularly the orange tortrix (*Argyrotaenia citrana* (Fern.)) and the holly bud moth.

All plots were examined on June 8. The number of injured tips on plants sprayed with TDE was very small when compared with the number on unsprayed plants near by. This was encouraging and may provide the added incentive needed to get more growers to remove unprofitable brush and trees that compete with and are often detrimental to the huckleberry, and to open up access or "jeep roads" through their holdings.

Life History.—Many larvae were collected from around Lakebay and Vaughan in early May, 1959 and brought to the Station. They were small and immature but apparently made a near normal growth in the rearing cages. However, experience with such insects as the orange tortrix had shown that when more mature larvae were collected they often yielded relatively more parasites, assuming they are parasitised, because they had been exposed to their enemies for a longer period of time.

Stands of "huckleberries" north of Shelton in Mason County were also found to be heavily infested with "tipworms" on May 6. This area was studied with more than usual thoroughness as the larvae approached maturity about the middle of June, and many of these more mature larvae were collected for the rearing cages. It was noted that when a larva was disturbed, it would quickly wriggle away, usually going backward, and would drop toward the ground, suspended by a silken thread. Many of the injured tips examined apparently had been abandoned in haste. If so, the larva on returning to the top of the plant to resume feeding apparently chose a different branchlet from that so hastily abandoned. Such behavior is reminiscent of that of larvae of the orange tortrix. Nearly 100% of the tips of some plants in this area had been injured.

Moths began appearing in the cages on July 1. A small

[1] Scientific Paper No. 1935. Project No. 1370. Accepted for publication June 10, 1960.

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[3] The name is a misnomer, since it is a blueberry, *Vaccinium* and not a true huckleberry, *Gaylussacia*.

ichneumonid wasp also began to appear. Cocoons of the wasps were found together with the remains of the larval skins of the "tipworms." Apparently, the parasite destroyed it's host in the last larval instar. The larvae pupated in the shelters where they had been feeding. A tachinid fly also appeared in one of the cages.

The area north of Shelton was visited on July 10 to compare the development of the "tipworms" under natural conditions with that of those in the cages. Not a single larva or pupa was found.

The appearance of moths in the cages ceased on July 15 almost as abruptly as it began. Nearly 100 specimens of a moth that resembled the holly bud moth had been preserved. However, they were smaller, the markings on the wings were less distinct and seemed to follow a different color pattern. In an effort to follow the development of the insect well into the fall, a few eggs were found. All were on the lower surface of leaves and most were down near the ground. No larvae were found.

IDENTIFICATION.—Moths and parasites were sent to the Entomology Research Division, U. S. Department of Agriculture, Beltsville, Maryland, for identification. The "tipworm" was identified as *Rhopobota naevana* (Hbn.) by Dr. J. F. Gates Clarke of the U. S. National Museum. This insect has been known for many years as the blackheaded fireworm of cranberry, *Vaccinium* sp. Hort. (Plank 1922). The species is generally credited with being closely related to the holly bud moth, but so far as is known, had limited it's feeding to the cranberry. It is easier to account for the insect's transferring from the cranberry to the "huckleberry" than from holly to "huckleberry" because both are members of the genus *Vaccinium*. Moreover, they are the only members of this genus in western Washington that are evergreen.

BIOLOGICAL CONTROL.—The tachinid fly was identified by C. W. Sabrosky as *Eulasonia* sp. Luella M. Walkley identified the ichneumonid wasps as *Horogenes* sp. There were 12 of these, suggesting that it may be of some importance in the natural control of the blackheaded fireworm. Only one specimen of a tachinid fly *Eulasonia* sp., appeared in the rearing cages. Franklin (1950) recorded another tachinid fly, *Nemorilla maculosa* (Meigen), as parasitic on the blackheaded fireworm in Massachusetts, and stated, "It (*N. maculosa*) is an important parasite of the black-headed and yellow-headed fireworms, and is the only parasite that has ever been reared from the former." Franklin's statement is of interest since the ichneumonid wasp, *Horogenes* sp., was reared from the Washington specimens in appreciable numbers. Crowley (1954) mentioned no parasitic insects, and Plank (loc. cit.) stated, "A fungus disease, which attacks the pupae in their cocoons on the old leaves beneath the vines, is responsible for the death of a large number, especially on old and badly infested bogs." He also stated, "Spiders and ladybird beetles kill a large number of the fireworm moths and larvae." This last statement is the only time parasites or predators were mentioned and is open to question.

HOST PLANTS.—A search of the literature has failed to reveal any reference to the blackheaded fireworm of cranberry feeding on any plant in America other than cranberry. Plank (loc. cit.) searched for other hosts, but found none. Crowley (loc. cit., p. 20) stated as recently as 1954, "Washington cranberry growers could probably exterminate the cranberry fireworm in a few years with a consistent spray schedule because of the absence of alternate hosts." The situation in Europe seems to be different. There, *Rhopobota naevana*, is credited with having several hosts, including holly, apple, plum, apricot and pear. However, according to Lucchese (1941), larvae on pear did not complete their development. He also considered it probable that a variety of *R. naevana* that prefers apple had developed.

SYNONOMY.—Carl Heinrich, who prepared the systematic description of *Rhopobota naevana* (Hbn.) for Plank's bulletin (loc. cit.), concluded that *R. vacciniana* (Pack.) was a synonym of Hübner's species *naevana*. He also concluded that the American species *Epinotia ilicifoliana* Kearfott should be referred to the genus *Rhopobota* as a variety of *R. naevana* (Hbn). Franklin (1948) used *R. vacciniana* (Pack.) (described from cranberry, a fruit that is indigenous to North America) as the technical name for the blackheaded fireworm of cranberry and stated, "The writer is not convinced that this species is the same as the European holly feeder, *R. naevana* (Hbn.), and hesitates to follow Heinrich (U. S. Dept. Agric. Bull. 1032, p. 45, 1922) and McDunnough (Memoirs, So. Calif. Acad. Sci. Vol. 2, p. 50, 1939) in this."

The present author has given much time to the study of the holly bud moth, *Rhopobota naevana ilicifoliana* during the past 20 years and, more recently, the blackheaded fireworm of cranberry. He has developed many of the same misgivings that appear to have troubled Professor Franklin whom he believes reached a valid conclusion.[4] The holly bud moth (Kearfott's *ilicifoliana*), as it develops in western Washington, is appreciably larger than the moth of the cranberry fireworm. It is usually darker, with a more pronounced color pattern, the browns being more distinct and better defined. The moth of *vacciniana* (Hübner's *naevana*, as presently accepted) is smaller and of a more somber gray with a less distinct color pattern. Moreover, larvae of *ilicifoliana* have never been found on anything but holly. Until recently, larvae of *vacciniana* were never found on anything but cranberry.

The overwintering eggs provide the most positive means of separating the two species in the field. With one exception, both insects attach their eggs to the underside of the leaves of the host plants. Eggs of *ilicifoliana* are often found on the smooth bark of the small branches of holly. Those of *vacciniana* have not been found on the pubescent branchlets of "huckleberry" nor have they been reported from the slender creeping stems of cranberry. Eggs of both species are flattened and disc-like, with partially raised and rounded centers, and each has a persistent opalescent sheen. They are of approximately the same size and usually elliptical in outline. A typical egg of *vacciniana* may measure approximately 0.66 mm. by 0.84 mm. Eggs of *ilicifoliana* are often circular in outline while those of *vacciniana* are always distinctly elliptical.

[4] The writer has presented his views to Dr. J. F. Gates Clarke, together with certain supporting evidence. Larvae from holly, "huckleberry" and cranberry have been reared to maturity in individual cages. Moths from these larvae together with the larval remains and empty pupal cases are being sent to Dr. Clarke for study.

It is in their respective colorations that the eggs of the two insects show the most striking differences. The freshly laid egg of *ilicifoliana* is of a pale amber color. In about 2 weeks it changes to a bright orange red surrounded by a narrow but distinct transparent border. The freshly laid egg of *vacciniana* is of pale lemon yellow color. The egg of *vacciniana* also changes color but the change is less noticeable, being from a lemon yellow to a light or medium amber, and the transparent border surrounding the egg is much less distinct than that surrounding the egg of *ilicifoliana*. Eggs of both insects retain their respective colors until shortly before hatching in late winter or early spring when the developing larvae, particularly their thoracic shields and head capsules, cause the eggs to darken appreciably. When placed side by side, the differences in the appearance of the overwintering eggs of the two insects, particularly those of coloration, are most striking. It is hoped that the observations reported in this paper will aid in clarifying the identity of the species discussed herein.

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Experiments for Early-Season Control of the Green Peach Aphid on Tobacco[1]

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ABSTRACT

Experiments were conducted for early-season control of the green peach aphid, *Myzus persicae* (Sulz.), on flue-cured tobacco with systemic insecticides employed before or at transplanting time, and aphid buildup studies were made following two field applications of several insecticides used in a preventive schedule for the control of other insects. Phorate and demeton applied in transplant water gave excellent control of the insect for a period of about 6 weeks. Phorate employed as a plantbed drench and as a post-transplanting foliage spray gave poor results. Dimethoate and Am. Cyanamid 18706 (*S*-(*N*-ethylcarbamoylmethyl) *O,O*-dimethyl phosphorodithioate) applied in transplanting water were less promising than phorate. The margin of plant safety of the four systemic insecticides, when used in transplanting water, is rather limited; thus commercial usage is not recommended.

As foliage sprays applied in a two application schedule, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), endrin, and Shell SD 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) greatly reduced the buildup of the green peach aphid. Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), TDE, Sevin® (1-naphthyl methylcarbamate), and Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-one) gave relatively poor results in preventing a buildup of the insect. In the dust form, Thiodan, Guthion, and endrin gave good control but Sevin, TDE, and Dipterex permitted moderate green peach aphid buildup.

The green peach aphid, *Myzus persicae* (Sulz.), has been recognized as an economic pest of flue-cured tobacco since 1946; however, its importance fluctuates from season to season. Chamberlin (1958) reviewed the history and status of this aphid as a pest of tobacco in the United States and concluded that a complex of climatic factors, host plants, and natural enemies influenced the intensity of infestations. Under Virginia conditions, the most damaging populations occur in tobacco fields where the insect becomes established early in the growing season and builds up to destructive numbers before it is checked by unfavorable weather conditions, biological agents, or artificial control measures.

Parathion and malathion are currently used for the control of the green peach aphid on flue-cured tobacco, but they are generally applied late in the season when the infestation demands treatment. Under these conditions, owing to the characteristic growth habits of tobacco, adequate coverage necessary for effective control with conventional equipment is difficult, and often the major damage has occurred before control measures are initiated.

Experiments were conducted from 1956 through 1959 with systemic insecticides applied before or at transplanting time for early-season control of the green peach aphid on flue-cured tobacco. During the last 2 years, aphid population buildup was studied following two

[1] Accepted for publication June 10, 1960.

[2] Acknowledgment is made to C. Y. Kramer, Statistical Laboratory, Virginia Agricultural Experiment Station, Blacksburg, Virginia, for the statistical analyses.

Table 1.—Early-season control of the green peach aphid with systemic insecticides.

Insecticide[a]	Ounces/ 50 Gal. Water[b]	Application Method	Average Number of Aphids per Plant on Days After Transplanting			
			21	28	35	42
		1956				
Phorate 90%, EC	1	Transplant water[c]	0.0	0.0	0.0	0.0
Phorate 40%, EC	2	Transplant water[c]	0.0	0.0	0.0	0.0
Demeton 25%, EC	2	Transplant water[c]	0.0	0.0	0.0	0.5
Check	—		17.6	38.1	42.9	51.3
		1957				
Phorate 90%, EC	1	Transplant water[c]	0.4	0.9	2.5	—
	4	Foliage after transplanting[d]	31.9	117.0	148.4	—
	1	Plantbed drench[e]	42.3	78.3	120.4	—
Check	—		53.3	191.4	175.7	—
		1958				
Dimethoate 46%, SC	1	Transplant water[c]	—	0.9	8.8	29.8
Am. Cyanamid 18706, 25% WS	2	Transplant water[c]	—	3.2	11.7	30.7
Check	—		—	33.1	24.1	34.6
		1959				
Dimethoate 46%, SC	2	Transplant water[c]	2.1	13.1	81.4	—
	4	Transplant water[c]	0.9	6.1	23.9	—
Check	—		18.3	89.4	269.7	—

[a] EC, emulsion concentrate; SC, soluble concentrate; WS, water soluble.
[b] Formulation.
[c] Applied at approximately 200 gallons per acre.
[d] 12 gallons per acre.
[e] 100 gallons per 100 square yards of plantbed area.

early-season treatments with the insecticides currently recommended for control of foliage-feeding insects and with several promising newer insecticides. Guthrie *et al.* (1956) reported that TDE, used as either a dust or spray, had some effect in reducing the aphid population and that endrin at a dosage of 0.29 pound per acre reduced aphid infestations appreciably. In Kentucky, Boush *et al.* (1956) found endrin to be moderately effective in reducing aphid populations on burley tobacco. The effect on the green peach aphid of some of the newer materials currently being investigated for the control of other tobacco insects has not previously been reported.

Procedure.—The tests with systemic insecticides were conducted in single-row plots of tobacco located on a Cecil sandy loam soil. The rows were 75 feet long, and each treatment was replicated four times in a randomized block design. Materials used in transplanting water were applied with a conventional hand transplanter, calibrated to deliver approximately 200 gallons per acre during the setting operation. Phorate, employed as a foliage treatment immediately after the plants were set in the field, was applied with a single-nozzle sprayer at 12 gallons per acre. The plantbed drench application was made 9 days before the transplanting date with a sprinkling can at the rate of 100 gallons per 100 square yards of plantbed area. During the second week after transplanting, the tobacco plants were artificially infested by brushing 5 to 10 green peach aphids on the bud of each plant to supplement the natural infestation. The effectiveness of the treatments was determined by counting the living aphids on 10 plants at random at weekly intervals for 3 or 4 weeks.

Field plots of tobacco utilized in experiments designed for the control of foliage-feeding insects were employed in the studies on buildup of the green peach aphid following two applications of an insecticide applied in a preventive schedule. A complete randomized block design was used in all tests and the treatments were replicated three or four times. The plots were four rows wide by 47 feet long and an untreated row was left between treatments. Alleyways, 6 feet wide, separated the replicates. The tobacco plants had a light infestation of the green peach aphid at the time they were removed from the plantbed for field setting.

The sprays were applied with single-nozzle knapsack sprayers. Applications were made on June 16 and July 1 in 1958 and June 5 and June 18 in 1959, at a dosage of 20 and 35 gallons per acre for the first and second applications, respectively. With the exception of Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) soluble powder and Sevin® (1-naphthyl methylcarbamate) wettable powder, all of the materials were liquid concentrates. The dusts were applied with rotary hand dusters. Applications were made on June 16 and June 30 in 1958 and June 6 and June 19 in 1959, at a dosage of 10 and 15 pounds per acre for the first and second applications, respectively. Green peach aphid population data were obtained 7 and 14 days after the second treatment by counting all living aphids on 10 plants selected at random from the two center rows of each plot. The multiple range and multiple F tests (Duncan 1955) were employed in the statistical treatment of the results and included a combined analysis of the two population counts.

Systemic Insecticides.—Table 1 gives the results obtained with the systemic insecticides using different methods of treatment. In the 1956 tests, two formulations of phorate and one of demeton were compared when used in the transplanting water. The aphid infestation in the check was only moderate; however, both materials gave excellent control for a period of 42 days after the plants were transplanted to the field. A gradual buildup occurred in the check, but the phorate-treated plants re-

mained free of aphids for the duration of the observation period, and there was no infestation in the demeton plot for 35 days. In 1957, the following phorate treatments were compared: (1) in transplanting water, (2) after transplanting as a foliage treatment, and (3) in the plantbed as a drench. The transplanting-water treatment was the only method which gave satisfactory control at the dosages used. Relatively poor results were obtained when phorate was employed as a post-transplanting foliage spray, and as a plantbed drench. In the 1958 and 1959 tests, dimethoate and Am. Cyanamid 18706 (*S*-(*N*-ethylcarbamoylmethyl) *O,O*-dimethyl phosphorodithioate) were used. The aphid population in the check was relatively low, but both materials gave good results for 28 days after the transplanting date. A moderate buildup had occurred after 35 days, and at the end of 42 days the aphid population in the treated plots was similar to that in the check. The green peach aphid population was larger in 1959 and dimethoate at the 4-ounce dosage was more effective than at the 2-ounce level in preventing a buildup of the insect.

Phorate and demeton applied by the transplanting-water method gave excellent early-season control of the green peach aphid; however, commercial usage is not recommended owing to the low margin of plant safety.

PHYTOTOXICITY.—Phorate and demeton in transplanting water at the dosages employed were responsible for slight necrotic spotting on the lower leaves of plants in some of the tests. The treated plants generally grew faster than the untreated and at the end of 6 weeks they were several inches taller. This difference in growth, however, was not apparent at the beginning of harvest. Both phorate and demeton at increased dosages were quite phytotoxic and resulted in severe plant stunting. Dimethoate and Am. Cyanamid 18706 at the rates employed in the 1958 tests resulted in no apparent phytotoxicity. In the 1959 tests, dimethoate at the 2-ounce dosage resulted in slight stunting and at the 4-ounce level the material was responsible for severe stunting with light mortality.

GREEN PEACH APHID BUILDUP STUDIES.—The materials used and the results obtained in the aphid population studies are presented in table 2. During each year in the spray tests, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) treatments showed less green peach aphid buildup than endrin, but the differences were not significant. In 1959 the buildup of the insect in plots treated with Shell SD 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) was similar to those treated with endrin. The Dipterex and TDE plots showed moderate aphid buildup, but the results were superior to those with Sevin and the check. Sevin and Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-one) plots were not significantly different from the check.

Among the insecticides used in the dust form, Guthion, Thiodan, and endrin all gave excellent results in preventing a buildup of the green peach aphid on tobacco.

Table 2.—Green peach aphid buildup on flue-cured tobacco following early-season treatment with insecticides applied as sprays or dusts for the control of foliage-feeding insects.

Insecticide[a]	Pounds/ 50 Gal. Water	Average Number of Aphids per Plant[b] on Days After Second Treatment			
		7	14	7	14
		1958		*1959*	
Sprays					
Guthion EC	0.375	0.0	0.0	0.0	0.3
Thiodan EC	.375	0.6	1.4	0.0	0.8
Endrin EC	.300	6.5	10.4	9.2	0.7
Shell SD 4402 EC	.300	—	—	11.7	1.0
Dipterex SP	.625	37.0	45.1	—	—
TDE EC	.625	44.9	49.4	—	—
Sevin EC	.313	54.0	50.1	—	—
Sevin WP	.85	—	—	87.5	55.4
Kepone EC	.50	—	—	57.9	50.0
Check	—	73.6	50.0	74.7	37.1
Dusts					
Thiodan 4%	—	0.0	0.5	0.0	1.5
Guthion 2.5%	—	0.1	1.0	—	—
Guthion 3%	—	—	—	0.0	0.7
Endrin 2%	—	0.0	1.5	—	—
Sevin 10%	—	12.7	38.5	34.7	14.7
TDE 10%	—	11.2	41.1	—	—
Dipterex 5%	—	43.3	57.8	47.5	38.5
Check	—	70.4	54.9	52.9	36.0

[a] EC, emulsion concentrate; SP, soluble powder; WP, wettable powder.
[b] Means paralleled by same line are not significantly different at the 5% level.

In the 1958 tests, the aphid population in the Sevin, TDE, and Dipterex treatments was similar to that of the check; but the Sevin treatment in the 1959 tests was superior to the Dipterex treatment and the check.

Weather conditions during July and early August of 1959 were favorable for continued green peach aphid buildup and moderate commercial damage to tobacco occurred in plots treated with Dipterex, Kepone, Sevin and the untreated check. Tobacco treated with endrin and Shell SD 4402 was lightly damaged. Insecticide treatment in accordance with a preventive schedule is not a recommended procedure for the control of insects on flue-cured tobacco. These studies indicate, however, that two properly timed early-season applications of Guthion, Thiodan, endrin or Shell SD 4402 reduce the probability of a buildup of the green peach aphid to destructive numbers.

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Organophosphorus and Carbamate Insecticides as Soil Treatments Against the Eye Gnat, *Hippelates collusor*, in the Laboratory[1]

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ABSTRACT

Twenty-four organophosphorus compounds, three carbamate insecticides and one chlorinated hydrocarbon insecticide (Thiodan®) have been screened in the laboratory as soil treatments against the eye gnat *Hippelates collusor* (Townsend). (Chemical definitions of proprietary materials are listed below.) Of the organophosphorus compounds, Co-Ral®, Bayer 25141, Experimental Nematocide 18133, Bayer 29493, GC-4072, Ethyl Guthion®, Trithion®, GC-3582, ronnel, G-30494, phorate, Bayer 22408, Methyl Trithion®, GC-3583, ethion, Guthion®, Di-Syston®, and Delnav® manifested a high degree of initial activity against the gnat. The first four compounds in this group had the greatest activity while Di-Syston and Delnav were less effective. Relatively moderate dosages of dimethoate and parathion were required for 90% reduction of the gnats. American Cyanamid Compound 18706, Dylox®, phosphamidon, and dicapthon proved effective only at higher dosages. Thiodan was ineffective at practical dosages.

Of the carbamate insecticides, AC-5727 was most efficient in reducing eye-gnat emergence from treated soil. Sevin® was effective, but only when applied in greater quantities, and m-t-butylphenyl N-methylcarbamate did not indicate appreciable activity at the rates used here.

CHEMICAL DEFINITIONS OF PROPRIETARY MATERIALS

AC-5727 (*m*-isopropylphenyl *N*-methyl carbamate)
American Cyanamid Compound 18706 (*S*-(*N*-ethylcarbamoylmethyl) *O,O*-dimethyl phosphorodithioate)
Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate)
Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate)
Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate)
Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate)
Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate 68%–70% and related materials)
Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate)
Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate)
Ethyl Guthion® (*O,O*-diethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate)
Experimental Nematocide 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate)
G-30494 (*S*-(2,5-dichlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate)
GC-3582 (diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate)
GC-3583 (diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate)
GC-4072 (2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate)
Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate)
Methyl Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate)
Sevin® (1-naphthyl methylcarbamate)
Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide)
Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)

Residual insecticides as soil treatments have been investigated recently for the control of *Hippelates* eye gnats. As a result of these investigations, only aldrin among the chlorinated hydrocarbon insecticides was found to have sufficient biological activity against eye gnats when applied at practical dosages (Tinkham 1953, Dow & Willis 1959). Subsequently, the effectiveness of this material against eye gnats in California was thoroughly investigated. The initial biological activity of aldrin applied to soil at two to three times the practical dosage was found to be moderate against *H. collusor* (Mulla *et al.* 1960). Continuing these studies the long-term effectiveness of aldrin, dieldrin, and heptachlor at moderate to high dosages against the eye gnat was further investigated in the field. None of these materials at the administered dosages yielded appreciable reduction of emerging eye gnats 7 to 9 months after treatment.

The initial biological activity of chlorinated hydrocarbon insecticides against *H. collusor* was investigated in the laboratory. Only four materials (Shell Development Company compound SD-4402,[2] endrin, aldrin and DDT) in this class of chemicals were found to manifest appreciable activity against the insect (Mulla 1960). Field trials with aldrin have already proved this material to have low residual activity. This leaves only three chlorinated hydrocarbon insecticides to be evaluated against eye gnats in the field. Field evaluation might further reduce the number of available compounds to be used in large-scale programs for the control of eye gnats.

The current investigations were initiated for the purpose of determining the initial biological activity of organophosphorus and carbamate insecticides against eye gnats. It is hoped that information developed here may lead to the discovery of certain effective materials with moderate or long residual trends in the soil.

A few of the organophosphorus insecticides also have been successfully employed for the control of a variety of soil insect pests (Howitt 1958, Hopkins *et al.* 1959). These materials were used in different ways, such as seed coating, side dressing, and furrow or broadcast application. Those organophosphorus compounds which manifest systemic activity have been employed for seedling pest control as seed coating and as granular or spray treatments (Reynolds *et al.* 1957). In most cases, the toxicants were located in the soil, and since *Hippelates* gnats breed mainly in cultivated farmland, the determination of the

[1] Paper No. 1225, University of California Citrus Experiment Station, Riverside, California. These studies were supported partially by a grant-in-aid from the Coachella Valley Mosquito Abatement District, Riverside County, California. Accepted for publication June 10, 1960.

[2] Proprietary materials are identified by their respective chemical definitions preceding the "References Cited."

effectiveness of such materials against the gnats is of importance. The application of organophosphorus and carbamate insecticides to soil for the control of agricultural pests would undoubtedly exert some pressure on the gnats breeding in the treated grounds. It is therefore obvious that elucidation of the effectiveness of such insecticides against gnats is important in predicting the role of these materials in gnat reduction. The application of an organophosphorus insecticide having greater activity against the gnats and having a short-to-moderate residual tendency in the soil is also desirable in certain situations where accumulation hazards of chlorinated hydrocarbon insecticides in farming land are eminent.

PROCEDURE.—The procedure for screening chlorinated hydrocarbon insecticides (Mulla 1960) was closely followed in the present studies. No major changes were introduced except that organic matter in the quantities indicated below was added to each half gallon (5.75 lbs.) of the treated soil: Brewer's yeast 14 gm.; whole wheat sifted flour 14 gm.; and alfalfa meal 28 gm.

The soil was sprayed with a De Vilbiss atomizer at the rate of 100 gallons of spray per acre. This amounted to spraying 1.16 ml. of the dilute spray over an 18-square-inch area of the one-half gallon of soil. The toxicants were applied at rates (lbs./acre) calculated on the basis of 1 acre soil 6 inches deep. The soil after drying was mixed thoroughly. The organic matter was added to the soil and the mixture was rolled and mixed again with a spoon. The mixture was divided into 5 equal portions, transferring each portion to a narrow-mouthed pint jar, thus every treatment being replicated five times. One hundred and forty ml. of water was added to the soil in each jar and 1 to 3 days later, each jar was seeded with 200 eye-gnat eggs. The eggs were obtained from the 6th to 34th generation of *H. collusor* colony established from wild eye gnat populations collected in Coachella Valley of southern California during the latter part of 1957. After addition of the eggs the jars were tightly covered with strainer cloth discs. The emerging gnats were counted at 2- to 3-day intervals and the reduction in gnat emergence was arrived at by comparison of the toxicant treatments with the control.

RESULTS AND DISCUSSION.—The organophosphorus insecticides screened in this study manifested varying degrees of initial activity against the gnat. There were several compounds which had to be applied at relatively high dosages to obtain appreciable reduction in gnat emergence from the soil.

Guthion®, Ethyl Guthion® (the same as Guthion, but the two methyl groups replaced by ethyl groups), and ethion manifested a good deal of activity. Guthion and ethion were more or less similar in their activity, but Ethyl Guthion manifested 3- to 4-fold more activity than did Guthion (table 1, A). Thiodan®, on the other hand, showed little activity and more than 30 pounds per acre of the toxicant were needed for obtaining 90% or more reduction of the emerging eye gnats.

The initial activity of dicapthon (American Cyanamid 4124) was very low. It was only at the highest dosage (16 lbs./acre) that any reduction in emergence was obtained (table 1, B). American Cyanamid 18706, dimethoate (American Cyanamid 12880) and parathion manifested moderate degrees of activity (see table 1, B). American

Table 1.—Reduction in eye-gnat emergence from soil sprayed with various dosages of various insecticides.

INSECTICIDE AND FORMULATION	TOXICANT, LB./6 ACRE-INCHES	AVERAGE NUMBER GNATS/JAR	PER CENT REDUCTION
	A. Experiment 1		
Guthion EC 1.5[a]	0.5	53	41
	1.0	19	79
	1.5	5	95
Control	—	90	—
Ethyl Guthion EC 2	0.1	52	47
	0.3	14	86
	0.5	6	94
	1.0	2	98
	1.5	0	100
Control	—	100	—
Thiodan EC 2	10	122	0
	20	68	43
	30	25	79
Control	—	119	—
Ethion EC 4	0.8	18	82
	1.0	12	87
	2.0	3	96
	3.0	2	99
Control	—	98	—
	B. Experiment 2		
Dicapthon EC 2	2	111	0
	4	112	0
	8	115	0
	16	48	57
Am. Cyanamid 18706 EC 2	2	120	0
	4	86	22
	8	74	33
	16	33	70
Dimethoate EC 4	1	54	51
	2	31	72
	4	18	84
Control	—	110	—
Parathion EC 2	2	110	4
	4	17	85
	6	2	98
	8	0	100
Control	—	116	—
E.N. 18133[b] Soln. (0.25 lb./gal.)	0.04	65	52
	0.08	21	85
	0.10	13	91
	0.50	5	97
Control	—	134	—
	C. Experiment 3		
Co-Ral EC 1	0.01	118	0
	0.02	100	15
	0.033	46	60
	0.05	13	89
	0.10	3	98

(continued on next page.)

[a] Emulsifiable concentrate formulation, containing 1.5 pounds toxicant per gallon. All the emulsifiable concentrate formulations of the toxicants are designated in this way. Soln., solution; WP, wettable powder.

[b] American Cyanamid Company.

Table 1.—(Continued)

Insecticide and Formulation	Toxicant Lb./6 Acre-Inches	Average Number Gnats/Jar	Per Cent Reduction
Bayer 25141 EC 2	0.05	29	75
	0.10	12	90
	0.20	2	98
	0.30	1	100
Bayer 29493 EC 2	0.05	73	38
	0.10	26	78
	0.20	2	98
Bayer 22408 Soln. (0.25 lb./gal.)	0.25	98	15
	0.50	36	69
	1.00	3	97
	2.00	0	100
	4.00	0	100
Control	—	117	—
	D. Experiment 4		
Ronnel EC 2	0.1	90	22
	0.2	54	53
	0.5	3	97
	1.0	0	100
Phosphamidon EC 4	2.0	106	9
	4.0	83	28
	8.0	75	35
	16.0	58	50
	24.0	33	72
Dylox Soluble Powder	10.0	117	0
	20.0	105	10
	30.0	54	54
Control	—	116	—
Phorate EC 4	0.1	128	5
	0.2	74	45
	0.4	23	83
	0.8	1	99
Di-Syston EC 2	0.2	139	0
	0.5	91	32
	1.0	30	78
	2.0	11	92
Control	—	134	—
	E. Experiment 5		
Delnav EC 4	0.6	103	10
	0.8	48	58
	1.6	3	97
	2.0	3	97
Trithion EC 4	0.1	86	25
	0.2	20	82
	0.4	2	98
	0.5	1	99
	1.0	0	100
Control	—	114	—
Methyl Trithion EC 4	0.1	159	0
	0.2	135	0
	0.3	83	30
	0.4	62	50
	0.8	9	93
	1.0	2	98

Table 1.—(Continued)

Insecticide and Formulation	Toxicant, Lb./6 Acre-Inches	Average Number Gnats/Jar	Per Cent Reduction
G-30494 EC 2	0.10	124	0
	0.15	125	0
	0.20	71	43
	0.40	13	90
	0.50	2	98
Control	—	124	—
	F. Experiment 6		
GC-4072 EC 4	0.05	105	0
	0.10	71	31
	0.15	17	84
	0.20	7	93
	0.40	1	99
GC-3582 EC 4	0.05	89	14
	0.10	98	5
	0.20	39	62
	0.40	4	96
GC-3583 EC 4	0.40	67	35
	0.60	23	78
	1.20	2	98
Control	—	103	—
	G. Experiment 7		
m-t-butylphenyl N-methyl carbamate soln.	10	52	58
	20	52	58
AC-5727 soln. (1 lb./gal.)	1	119	4
	2	96	23
	4	75	40
	8	31	79
	10	15	89
Sevin 50% WP	10	70	45
	20	11	91
	30	0	100
Control	—	125	—

Cyanamid Compound Experimental Nematocide 18133 was rightly effective and gave 90% reduction in gnat emergence at the 0.1 lbs./acre rate.

The basic structural formulas of dicapthon and parathion are essentially the same, except that the former compound has a chlorine atom attached to the nitrophenyl ring and has two methyl groups replacing the two ethyl groups of parathion. It has been observed that the replacement of ethyl groups by methyl groups, in Ethyl Guthion to yield Guthion and in Trithion® to yield Methyl Trithion®, resulted in reduced effectiveness of the methyl analogues. It is assumed that the methyl analogues break down rapidly in the soil.

Dimethoate and American Cyanamid 18706 have similar structures. The substitution of methyl and ethyl groups on the N-carbamoyl group yields dimethoate and American Cyanamid 18706, respectively.

The former compound, however, was found to be more effective against the gnat in the soil than the latter. Here

the methyl analogue proved to have more activity than the ethyl analogue.

Co-Ral®, Bayer 25141 and Bayer 29493 all manifested enhanced activity (table 1, C). Less than 0.2-lb./acre rates of these materials were needed to obtain 90% or more reduction of the emerging gnats. Bayer 22408 was not as effective as the former three materials, but still manifested a good deal of activity.

The activity of phosphamidon and Dylox® against the gnat was very low. Ronnel, on the other hand, was highly effective. Phorate and Di-Syston® also manifested a good deal of activity (table 1, D), although the former material showed greater activity than the latter.

Delnav®, Trithion, Methyl Trithion and Geigy Chemical Company Compound G-30494 were found to be highly effective (table 1, E). Trithion and G-30494 were more effective than Delnav and Methyl Trithion. The activity of Trithion was about three times that of Methyl Trithion as measured at the LD50 and LD90 levels. Substitution of ethyl groups in Trithion with methyl groups to give Methyl Trithion, resulted in reduced toxicity of the latter compound.

Three experimental compounds from General Chemicals were evaluated. Of the three materials, compounds GC-4072 and GC-3582 proved more effective than compound GC-3583 (table 1, F). Compound GC-4072 proved to be 1½ to 2 times as effective as compound GC-3582 and approximately 4 times as effective as compound GC-3583. Compound GC-4072 is a structural isomer of compound GC-3583 with the two chlorine atoms at positions 2 and 4 on the phenyl ring in the former compound and at positions 2 and 5 in the latter. Compound GC-3582 is an analogue of compounds GC-3583 and GC-4072. The former compound has a dichlorovinyl group while the latter two have monochlorovinyl groups instead.

Of the three carbamate insecticides evaluated, compound AC-5727 proved to be the most toxic one against the gnat (table 1, G). Sevin® was the next material with appreciable activity. An experimental material (*m-t*-butylphenyl N-methyl carbamate) did not manifest appreciable activity.

All the materials with the exception of *m-t*-butylphenyl N-methyl carbamate, evaluated in this study are arranged in their decreasing order of effectiveness in table 2. The initial LD50 and LD90 level rates in pounds per 6 acre-inches of soil are presented. From this table it is obvious that Co-Ral is the most effective of all the materials and Dylox and phosphamidon the least effective ones. The effective rates both at the LD50 and LD90 levels are relatively low for all the materials up to and including Delnav in this list. Following Delnav, the next three materials in the list (parathion, dimethoate, and AC-5727) manifested moderate activity, while Sevin and Am. Cyanamid 18706 showed lower activity. The remaining materials in the list are believed to have very low activity and for economic reasons may not be suitable for gnat control.

The dosage mortality lines on a log-probit scale for all the materials evaluated in this study except *m-t*-butylphenyl N-methyl carbamate and dicapthon, are presented in figures 1 and 2. Most of the lines have steep slopes. Parathion, Sevin, Thiodan and Dylox, although less active, have lines with steep slopes. Dimethoate and phosphamidon and Am. Cyanamid 18706 have very flat dosage mortality lines. Ethyl Guthion and Bayer 25141, AC-5727 and ethion, also have lines with slightly flat slopes. The remaining materials all have lines with relatively moderate and steep slopes.

Table 2.—Initial effectiveness of organophosphorus and carbamate insecticides and of Thiodan against the eye gnat, *Hippelates collusor*.

INSECTICIDE	POUNDS TOXICANT PER 6 ACRE-INCHES	
	LD_{50}	LD_{90}
Co-Ral	0.030	0.052
Bayer 25141	0.025	0.096
E.N. 18133	0.041	0.092
Bayer 29493	0.060	0.131
GC-4072	0.118	0.188
Ethyl Guthion	0.110	0.370
Trithion	0.136	0.250
GC-3582	0.184	0.310
Ronnel	0.178	0.368
G-30494	0.210	0.400
Phorate	0.240	0.485
Bayer 22408	0.400	0.740
Methyl Trithion	0.400	0.840
GC-3583	0.445	0.805
Ethion	0.380	1.260
Guthion	0.60	1.30
Di-Syston	0.74	1.60
Delnav	1.00	1.64
Parathion	3.35	4.80
Dimethoate	1.00	5.00
AC 5727	4.00	10.20
Sevin	10.50	20.50
Am. Cyanamid 18706	9.50	28.00
Dicapthon	12.00	—
Thiodan	21.50	33.00
Dylox	29.00	43.00
Phosphamidon	16.00	53.00

Studies on the long-term effectiveness of these organophosphorus and carbamate insecticides as soil treatments against the gnats are in progress. Since materials with moderate to long-term longevity are sought, these studies would undoubtedly eliminate most of these compounds. From the chemical nature of these compounds, very few are expected to manifest biological activity over prolonged periods of time (such as 6 to 12 months) at economical dosages. Nevertheless, the information presented here on the initial biological activity of these compounds against *H. collusor* (the predominant species of eye gnats in the Southwest) increases our knowledge with regard to the use of various insecticides for the control of these pests of public health importance under a variety of farming conditions.

The chemical definitions of proprietary materials mentioned in this article are as follows:

AC-5727 (*m*-isopropylphenyl *N*-methyl carbamate)

American Cyanamid Compound 18706 (*S*-(*N*-ethylcarbamoylmethyl) *O,O*-dimethyl phosphorodithioate)

Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate)

Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate)

Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate)

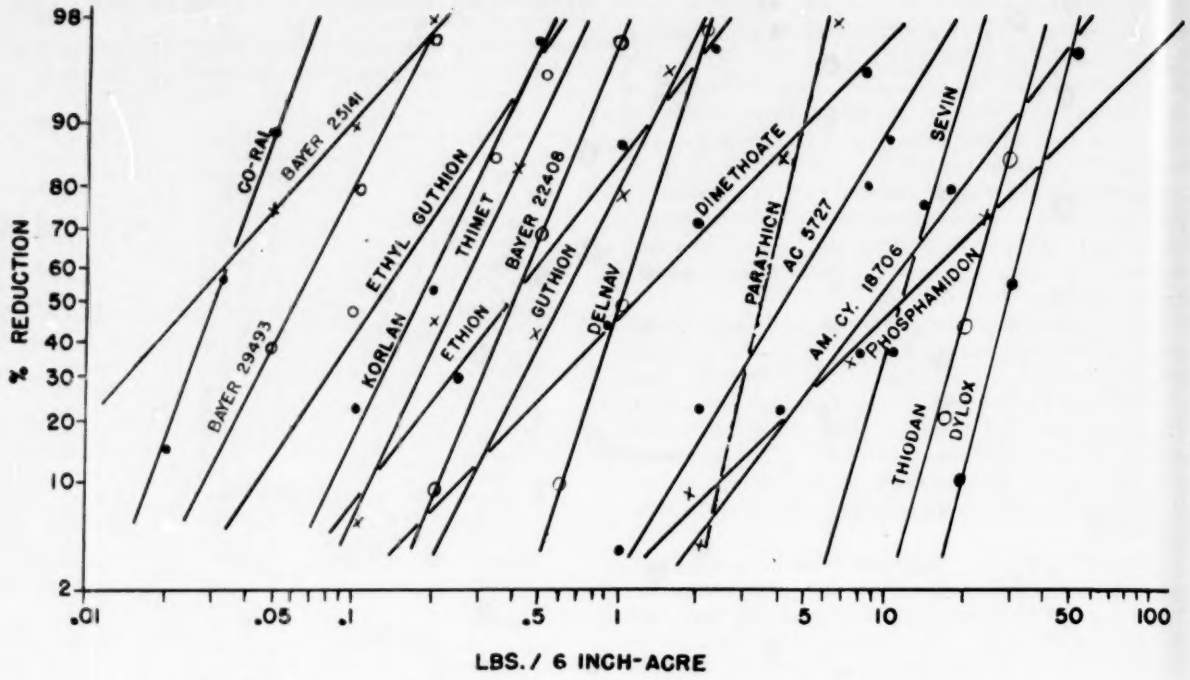

FIG. 1.—Dosage mortality lines expressing the initial effectiveness of various insecticides in soil against the eye gnat, *Hippelates collusor*.

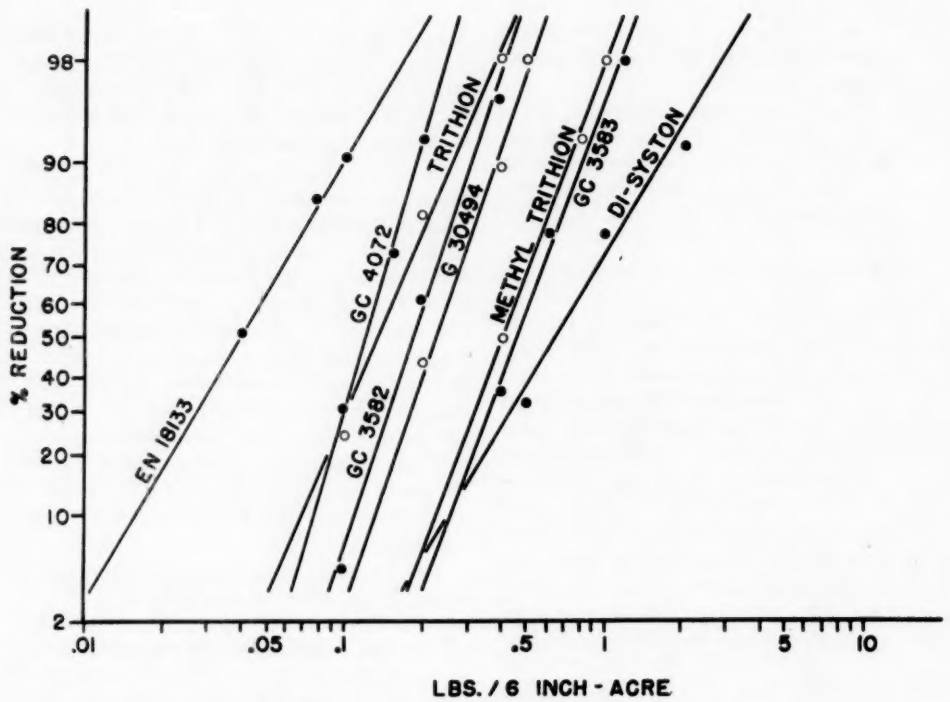

FIG. 2.—Dosage mortality lines expressing the initial effectiveness of various insecticides in soil against the eye gnat, *Hippelates collusor*.

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate)
Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate 68%–70% and related materials)
Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate)
Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate)
Ethyl Guthion® (*O,O*-diethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate)
Experimental Nematocide 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate)
G-30494 (*S*-(2,5-dichlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate)
GC-3582 (diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate)
GC-3583 (diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate)
GC-4072 (2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate)
Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate)
Methyl Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate)
Sevin® (1-naphthyl methylcarbamate)
Shell Development Company Compound SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan)
Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide)
Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)

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Drosophila Control in Tomato Fields[1]

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ABSTRACT

Insecticidal sprays were tested for the field control of Drosophila oviposition—principally that of *Drosophila melanogaster* Meig.—in tomato fruits during 1955 and 1957. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) applied at the rate of one-half pound per acre gave a satisfactory reduction of Drosophila activity. Wettable powder, emulsifiable concentrate and a granular formulation of Diazinon were compared and found to be equally effective at the rate of one-half pound per acre. The granular formulation exhibited long residual action and there appeared to be some cumulative effect from repeated applications. Malathion applied to small plots and in commercial plantings was only effective for 2 to 3 days and was followed by a very rapid fly buildup. Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) had somewhat the same weakness as malathion—short residual action. Ronnel and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) were tested during 1957 only and compared favorably with Diazinon. Hydrolyzed yeast did not prove to be an added attractant to the flies when used alone.

Drosophila—principally *Drosophila melanogaster* Meig.—is not a new pest in tomato fields of western New York. In the past, it has been controlled only around and in the processing plants. Increased infestations in the past few years and more stringent enforcement of insect contamination regulations have forced tomato processors to recognize the tomato field as one of their major sources of contamination. The present investigation deals only with the field phase of the problem.

Considerable effort has been devoted to investigation of the field control of Drosophila in California and New Jersey where the problem has been acute. In New Jersey, Pepper and others (1953) attempted to control Drosophila oviposition in the field with insecticide sprays but the results were only fair to unsatisfactory. The most promising method of control was obtained by spraying the baskets of harvested tomatoes with a 2% pyrethrin formulation. This treatment appeared to prevent oviposition for a period of 24 hours.

Michelbacher and others (1953) in California tested several insecticides as aerial applications to tomatoes. A dieldrin spray of 1 pound of actual per acre and a 10% DDT dust showed considerable promise. Further tests conducted during 1953 by Michelbacher & Middlekauff (1954) showed that an aerial spray of 1 pound of dieldrin per acre gave excellent control of oviposition for about 15 days while DDT was ineffective. These authors cautioned the reader that the excellent results obtained with dieldrin were obtained during cool weather which was generally

[1] Journal Paper No. 1220, New York State Agricultural Experiment Station, Geneva, June 10, 1960. Accepted for publication June 15, 1960

unfavorable for Drosophila. A spray of malathion+sugar applied by hand to small plots gave control of oviposition, but the results were variable and inconclusive.

Steiner (1954) working in Hawaii obtained good control of the oriental fruit fly (*Dacus dorsalis* Hendel) and the melon fly (*Dacus cucurbitae* Coq.) on various tropical fruits by using bait sprays containing ½ to ¾ pound of malathion and 1 pound of hydrolyzed yeast. While these fruit flies are not closely related to Drosophila, the success obtained with this bait spray provided the stimulus for investigating its possible applicability to Drosophila control in the field. This work was initiated in 1955.

It was decided at the outset that the organic phosphate types of insecticides would be stressed for Weiner & Crow (1951) and Bartlett (1952), as well as others, had demonstrated that Drosophila could acquire resistance to DDT. Crow (1954) claimed that Drosophila once resistant to DDT would not revert appreciably to DDT susceptibility, even 3 years after the DDT pressure was removed. On the other hand, King (1954) claims that this type of inheritance (*e.g.*, DDT-resistance) was usually unstable once the producing force was removed. Thus, despite the differing opinions at that time, it seemed wiser to overlook the chlorinated hydrocarbon type of insecticides in view of the potential resistance problems that could be encountered at a later date.

METHODS.—In 1955, several insecticides were tested as sprays in plots of tomatoes for the control of oviposition on tomato fruits by Drosophila. Three plots, 200′×36′ (6 rows), of Red Jacket tomatoes were used. Plots were separated 300 feet from each other in an attempt to minimize the effect of reinfestation from adjacent plots. The untreated plot was placed in the center of the experimental layout to equalize as much as possible the chances of reinfestation from it. Cultural practices up to harvest time were normal for western New York. The crop was not harvested except to remove those tomatoes sampled daily for recording adult activity. All sprays were applied with a tractor-mounted sprayer equipped with a single-row boom (5 nozzles per row). Sprays were applied at the rate of 120 gallons of spray per acre at a pressure of 80 p.s.i. Plant diseases were controlled with maneb (manganese ethylenebisdithiocarbamate) applied at the rate of 4 pounds of the 70% formulation per acre. Fungicide applications were made on a 10- to 14-day schedule. Insecticide treatments were applied on August 9, 17, 24, 31, September 6, 14, 21, 28 and October 5. One pound of yeast hydrolysate (enzymatic) (produced by Nutritional Biochemicals Corp.) was added to the sprays (applied alone to the untreated plot) up to and including the application of September 14.

Oviposition activity of the Drosophila flies was measured with a modification (four, 1-inch slits per trap tomato) of the slit-tomato technique described by Michelbacher (1954). Ten stations were established at random in the two middle rows of each plot. Stations were staked and moved within these two rows at frequent intervals. One trap tomato was placed in the shade of the vines at each station for each 24-hour period. Tomatoes were collected and replaced between the hours of 10 a.m. and 12 noon. Collected tomatoes were stored in 1-gallon paper ice cream containers which were equipped with screened vents, drain and a hardware cloth rack to keep the tomatoes off the bottom of the cage. Cages were stored in a greenhouse maintained at a temperature of 80° F. Once all the larvae had pupated, the pupae were counted and then destroyed. When counting could not be done immediately, the cages were stored at 32° F. until it was convenient to do so. Daily counting of the eggs deposited in the slits is perhaps a more desirable technique but it proved impracticable for our particular labor situation.

A test for the control of Drosophila oviposition in a commercial tomato field was conducted during 1955 at Alton, New York. A 15-acre field of Red Jacket tomatoes bordered on three sides by pear and apple orchards was used. A 4-acre plot (35 rows×995′) on the north side of this field was sprayed on August 15 with malathion at the rate of 0.5 pound of the toxicant per acre and on September 1 with 1 pound. One pound of partially hydrolyzed yeast per acre was included in each spray application. Sprays were applied with a potato sprayer equipped with a 7-row boom (5 nozzles per row) at the rate of 200 gallons per acre at a pressure of 350 p.s.i. Ten sampling stations were randomly placed in the treated plot and also in the adjoining 4 acres of untreated tomatoes. Sampling was done between 1 and 2 p.m. each day. Preparation and handling of trap tomatoes was as described previously.

In 1957, nine plots (150′×5 rows) of Red Jacket tomatoes were planted in a 10-acre field. Plots were isolated from each other with plantings of other crops much as in 1955. Sprays were applied with the same machine used in 1955 but applied at the rate of 46 gallons per acre. The granular formulations were applied with a hand-drawn Gandy fertilizer spreader. Maneb was applied to all plots at 10-day intervals to control plant diseases. Sampling for Drosophila activity was as described in the 1955 tests. In 1956, the cages were changed from ice cream containers to 10-pound cherry pails as these containers were less susceptible to contamination through leaks as well as being reusable.

RESULTS AND DISCUSSION.—Drosophila activity in 1955, as determined by oviposition in trap tomatoes, increased rapidly during late August and early September with the peak occurring on September 6 (table 1). The two cold wet days of September 7 and 8 reduced oviposition from the season's peak of 543 per tomato, September 6, to about 60. The remainder of September and early October were characterized by intermittent periods of cold weather which generally were unfavorable for Drosophila oviposition and are reflected in low counts in the untreated plots. Such variation made field evaluation of the insecticides more difficult.

Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) applied at 0.5 pound per acre was effective for about 5 days followed by increased Drosophila activity but much less than in the untreated plot. Reducing the concentration of Diazinon to 0.25 pound per acre on August 31 did not materially affect control efficiency until the second week (September 6 to 13) at which time the residual effectiveness was reduced to about 2 days. This residual effectiveness might have been even less since comparatively cool weather prevailed on September 7 and 8 and the flies were rather inactive. When the concentration of Diazinon was reduced to 0.125

pound per acre (September 14), the residual activity was still about 2 days. As might be expected, increasing the concentration to 0.5 pound per acre on September 21 did not increase the efficiency to that level obtained in August; residual effectiveness was only 3 days as compared with 5 at that time. It was evident from the early results that malathion was not effective at 0.5 pound per acre. Increasing the concentration of malathion to 1 pound on August 31 resulted in increased control effectiveness, but it was not until the week of September 6 that the degree of control was satisfactory. The poor ovipositional period of September 7 and 8 again may have been contributing a greater effect here than the malathion treatment. On September 14, parathion at 2 pounds per acre was substituted for the malathion treatment and proved to be very effective although the dosage was excessively heavy. Phosdrin® (1 methoxycarbonyl-1-propen-2-yl dimethyl phosphate) applied at 0.25 pound per acre was substituted for the parathion treatment on September 21. Effective control was obtained for 3 days at which time Drosophila activity immediately increased. Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), DDT and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) were tested during the period September 28 to October 11, but activity in the untreated plots was too erratic to give a reliable test.

Drosophila activity in the commerical tomato planting at Alton, New York, became very heavy on August 20, or nearly 2 weeks earlier than at Geneva (table 2). This field was bordered on three sides by apple and pear orchards which in all probability played a major role in both severity and earliness of the infestation. The application of one-half pound of malathion on August 15 gave excellent control when the population was low and inactive. The 1 pound of malathion applied on September 1 gave practical control for only 1 day although there was a considerable reduction over the untreated for 3 days. While this test may have had a more severe insect infestation owing to the unfavorable location of the tomato field—a location often encountered in the tomato-growing areas of New York—this lack of residual activity with malathion makes its use impractical, particularly in view of the more promising results obtained with Diazinon at Geneva.

There were only 2 weeks (September 12 to 27) during 1957 when Drosophila activity was heavy enough for adequate testing of field control programs (table 3). The results obtained with applications of the hydrolyzed yeast alone closely parallel, with a few deviations, those obtained in the untreated plots. This agreed with impressions and inconclusive evidence obtained in 1955 and 1956. It was perhaps illogical to postulate that a yeast spray would act as an attractant in a tomato field for there are such large amounts of yeast and fermentation products produced naturally in tomato fields during harvest time. Unsatisfactory control was obtained with one-fourth pound of Diazinon during both weeks although it was more accentuated during the second week. This confirmed the writer's opinion of this dosage obtained during 1955 when low activity for 2 days after treatment confused the results. The ½-pound dosage of Diazinon was highly effective for about 3 days during the first week and even more effective during the second week. This situation also was experienced during 1955 and tends to indicate that there is a cumulative effect of treatments. There did not appear to be any significant difference between the wettable powder and the emulsifiable concentrate formulations during the first week although there is more deviation than can be explained during the second week. The

Table 1.—Control of oviposition by Drosophila in tomato fields with insecticides. Geneva, N. Y. 1955.

Treatment[a]	Toxicant per Acre (lb.)	Average Number Pupae per Tomato[b] (days from treatment) 0	1	2	3	4	5	6
Aug. 17–23								
Diazinon	0.5			0	3	Tr	Tr	7
Malathion	0.5			0	0	12	126	66
Untreated	—			Tr	Tr	16	43	79
Aug. 24–30								
Diazinon	0.5	4	2	Tr	16	7	10	42
Malathion	0.5	61	10	77	84	46	147	161
Untreated	—	35	50	46	143	98	93	154
Aug. 31–Sept. 5								
Diazinon	0.25	39	3	3	5	3	47	
Malathion	1.0	116	51	41	60	81	306	
Untreated	—	146	232	277	263	204	290	
Sept. 6–13								
Diazinon	0.25	49	1	3	25	31	119	52
Malathion	1.0	98	2	4	45	20	113	142
Untreated	—	543	55	62	242	250	124	91
Sept. 14–20								
Diazinon	0.125	78	4	Tr	25	39	32	39
Parathion	2.0	158	5	1	4	19	18	46
Untreated	—	167	54	84	120	121	130	231
Sept. 21–27								
Diazinon	0.5	36	1	1	2	53	12	2
Phosdrin	0.25	118	4	4	4	117	10	3
Untreated	—	132	56	110	87	186	19	20
Sept. 28–Oct. 4								
Diazinon	0.25	3	5	4	22	2	6	15
Dipterex	0.50	28	1	1	17	Tr	1	32
Untreated	—	24	19	37	62	14	50	81
Oct. 5–11								
DDT	0.50	13	13	46	11	63	52	99
Guthion	0.50	33	3	28	8	35	52	43
Untreated	—	81	37	46	57	224	150	168

[a] Treatments applied August 17, 24, 31, September 6, 14, 21, 28 and Oct. 5. 2 lbs. of yeast hydrolysate applied to all plots except Sept. 21, 28 and Oct. 5.
[b] Rounded to nearest whole number. Tr = trace or less than 1.

Table 2.—Effectiveness of malathion spray in controlling oviposition by Drosophila flies on tomatoes in a commercial planting. Alton, N. Y. 1955.

Days After Treatment	Average Number Pupae per Tomato[a] August 15–23 Malathion[b] (0.5 lb.)	Untreated	Sept. 1–6 Malathion[c] (1 lb.)	Untreated
1			16	115
2	1	16	61	341
3	1	9	178	461
4	1	19	429	589
5	1	393	391	515
6	39	27		
7	287	463		

[a] Rounded to nearest whole number.
[b] Applied August 15.
[c] Applied September 1.

Table 3.—Sprays to control *Drosophila* sp. in tomato fields. Geneva, N. Y. 1957.

Treatment[a]	Toxicant per Acre (lb.)	Average Number of Drosophila Pupae per Tomato[b] (days after treatment)							
		0	1	2	3	4	5	6	7
Sept. 12–19									
Untreated	—	18	50	56	43	67	27	49	51
Yeast	2.0	11	46	21	45	58	31	50	47
Diazinon, w.p.	0.25	31	17	17	10	24	32	47	53
	0.5	27	4	1	7	18	5	21	13
Diazinon, e.c.	0.5	10	3	5	7	18	20	28	48
Diazinon, granules	0.5	28	3	1	Tr	25	8	14	26
Ronnel, w.p.	1.0	37	1	Tr	6	15	15	21	25
Ronnel, granules	1.0	30	Tr	3	5	5	13	22	22
Dibrom, w.p.	1.0	13	0	3	4	11	14	7	12
Sept. 19–27									
Untreated	—	51	94	97	119	80	57	56	94
Yeast	2.0	47	88	100	136	107	59	56	136
Diazinon, w.p.	0.25	53	29	35	59	57	46	22	103
	0.5	13	Tr	Tr	1	4	3	1	37
Diazinon, e.c.	0.5	48	2	8	9	16	19	7	18
Diazinon, granules	0.5	26	3	0	Tr	Tr	1	Tr	2
Ronnel, w.p.	1.0	25	1	Tr	12	2	14	Tr	37
Ronnel, granules	1.0	22	17	4	11	7	1	1	1
Dibrom, w.p.	1.0	12	5	2	16	13	12	28	39

[a] Treated Sept. 12, 19.
[b] Rounded to nearest whole number. Tr = trace or less than 1.

results with the granular formulation (tobacco stems) closely parallel those obtained with the wettable powder. During the second week, the granular formulation gave outstanding results. This formulation of Diazinon had excellent physical qualities, sifting down to the lower leaves and soil under the plants. Since the adults tend to congregate under the plants where it is shady, this formulation has distributive advantages not available to the sprays. Ronnel and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) gave results comparable to Diazinon during the first week but were a little erratic during the second week. These and Dibrom certainly are promising for future investigations.

The Drosophila fly poses a problem to New York processors in three areas: 1) in the field, where eggs are laid in fresh cracks in the tomatoes; 2) in the harvested tomatoes, either in the field or en route to the factory where rough handling through picking and transporting produces the cracks necessary for oviposition; 3) at the factory, including the yard, unloading platforms and within the plant proper. A spray program that decreases the populations of Drosophila in tomato fields and, hence, the eggs on or in tomatoes seems to be possible and practical. The period when control is necessary, of course, will vary with the season or weather, for this insect's activity is limited very severely by these factors (Michelbacher & Middlekauff 1954). Under New York conditions, fields appear to be susceptible to infestations any time from the third week of August until late September. Thus, a control program would be based on weekly applications for a maximum of perhaps 4 weeks. This does not protect harvested tomatoes en route to the factory, but the work of Stombler and others (1957) would appear to offer a program that could well supplement the field control program.

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Mass Production of Screw-Worms (*Callitroga hominivorax*) for the Eradication Program in the Southeastern States[1]

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Abstract

From January 1958 to October 1959 slightly more than 3¾ billion pupae were produced, utilizing approximately 6½ million pounds of horse and whale meat, during the screw-worm eradication program conducted in the Southeastern States, by the procedures described. The number of grams of eggs obtained from the stock colony and the grams required to meet the weekly fly production quota; the amount of horse and whale meat utilized in producing the minimum of 50 million flies weekly required for field releases; and the number of pupae produced in relation to the number of flies released are shown graphically.

In nature screw-worms (*Callitroga hominivorax* (Cqrl.)) develop only in the living tissues of warm-blooded animals, causing serious injury and often death unless some form of treatment is applied. The annual damage in livestock alone has been estimated to be about 10 million dollars in Florida and about 20 million in the Southeastern States. The successful completion of the Florida-Federal

[1] Accepted for publication June 13, 1960.

[2] The three phases of mass production were under the immediate supervision of assistants H. M. Brundrett, fly colony; Gerald Diamant, rearing larvae; Frank H. Dudley, handling pupae.

screw-worm eradication program has eliminated this loss, although a field inspection force is maintained in the States concerned. The completion of the program through the release of sexually sterile males has stimulated interest in similar programs for other insects. The object of this paper is to set forth the procedures used in producing at Sebring, Florida the millions of screw-worm flies necessary for field releases, with the hope that the methods might be helpful in the development of other large-scale programs.

For a number of years the larvae of this obligatory parasite had been produced in adequate numbers on an artificial medium (Melvin & Bushland 1941) consisting of lean ground meat, blood, water, and formaldehyde. Eggs were obtained by confining several gravid females in a shell vial with a small piece of lean meat held at 95° to 100° F. for 2 to 3 hours. The cultures of larvae were grown in ordinary wash tubs or bun trays in a room or cabinet heated to the same temperature. This method was adequate for producing the few thousand insects required for experimental use and large field tests, but was inadequate for sustained production of the 50 million each week required for the eradication program.

Laboratory experiments (Bushland & Hopkins 1951, 1953) demonstrated that screw-worm flies could be made sexually sterile by irradiation of the pupae with X-rays and gamma rays. In a field test Baumhover *et al.* (1955) released 400 sterile males per square mile weekly over the 170 square mile island of Curacao in the Netherlands Antilles and eradicated the insect from the area. The procedures for mass production of screw-worm larvae (Graham & Dudley 1959) by which 2 million flies per week were produced for 15 consecutive weeks and the rearing medium formula developed by Melvin and Bushland were basically the cultural procedures used for mass production of flies for the eradication program. Not all these procedures were strictly adhered to. Some of them were varied slightly to meet or overcome a specific situation, but it is only through experience gained from actually rearing and caring for the insects that the worker is able to vary these procedures and rearing media formula without getting into serious difficulties. Detailed information regarding descriptions and specifications of the various types of equipment and controls are not presented in this paper. For brevity, only reference to size and numbers are given.

PROCEDURES.—*Fly Colony Maintenance.*—The fly colony occupied several rooms of varying size. The holding room 19.5 feet wide×145 feet long×10 feet high, was regulated to 80° F. and 50% to 60% relative humidity. It was maintained in complete darkness, except in emergencies and at one end where the flies were induced to oviposit. For fly security reasons, entrance into and out of the fly colony was through a series of three vestibules so that all persons and materials leaving the area was inspected to assure that no live fly escaped.

To perpetuate the fly colony and to assure adequate eggs to maintain a minimum production of 50 million flies per week for field releases required that approximately 5% of the pupae produced be diverted into the colony from the production line. The pupae destined for the fly colony were taken from normal production and were no better or worse than those irradiated for release. They were earmarked and set aside in the pupae holding room where they remained until they were about 5½ days old. They were taken from the pupae holding room and placed in the fly cages 24 to 36 hours before they were to emerge.

The stock fly colony consisted of flies held in 54 aluminum screen and metal cages, 42 inches wide×71 inches long and 42¾ inches high. The cages were set up in a rotating schedule so that during each 8-hour shift, 2 cages were filled with pupae and put in operation, 2 cages of flies were induced to oviposit and after oviposition, the contents and flies of the 2 cages were destroyed. By following this schedule six cages of 8-day-old flies were available for obtaining eggs each day, a number adequate for maintaining the desired 50 million flies per week for field release and colony perpetuation.

Each fly cage was usually stocked with 6 liters of pupae in three screen-bottomed trays 18×26×2 inches, about 60,000 flies, but if there was any indication that the emergence might be below normal an additional 1½ liters of pupae were added. Before the pupae were introduced the floor of the cages were lined with heavy wrapping paper and from seven overhead rods paper toweling was hung to near the floor to serve as resting places for the flies and to absorb fecal matter. Food for the adult flies was provided in two aluminum bun pans 18×26×1 inches containing a mixture of equal parts of ground lean horse meat and honey. Additional honey and water was provided in pint and quart jars made into fountains by screwing onto them the glass bases of watering devices used by poultrymen. After the jars were filled they were inverted, and oat hulls were sprinkled lightly on the honey oozing from the fountain and absorbent cotton was placed about the water fountain. Oat hulls were also sprinkled lightly over the meat-honey mixture to provide footing for the flies. The two pans were placed on the bottom center line of the cage. The honey and water fountains were placed alternately on each side of the cage. After the three trays containing pupae were placed on the overhead rods in the cage, the door was securely closed and not opened again until the flies had been induced to oviposit. After oviposition was completed, the cages were held in a cooling room until the flies became immobilized. The cages were then opened and the immobilized flies, pupae cases, paper toweling, etc., were removed and placed in paper bags for destruction.

Oviposition.—The pupae were 6 days old when placed in the cages, and emergence occurred within 24 to 48 hours. The flies were aged for 8 days before being induced to oviposit. Six cages containing pupae were set up and put in operation and 6 cages of flies were induced to oviposit then destroyed each 24 hours (three 8-hour shifts) resulting in a total of 54 cages in a rotating schedule.

When it was desired to obtain eggs two cages of 8-day-old flies were moved to one end of the stock colony holding room, separated from the other cages by a curtain. An egging device 12×66×2 inches made of aluminum and containing an electrical heating coil, was introduced into the cage through a stockinette sleeve located in the lower portion of the cage door. The oviposition medium, placed about ½ to ¾ inch thick in the egging device, was prepared by thoroughly mixing one-half pint of an attractant with

approximately 10 pounds of lean ground horse meat. The attractant was prepared by incubating the juices extracted from ground beef heart at 98° F. for 3 days or 20 grams of blood albumin in 400 cc. water for 10 to 12 days. A series of 15 wooden cells 4 inches square, in each of which was a 7½-watt light, were placed on the oviposition medium. The temperature of the oviposition medium was thermostatically controlled at 98° to 102° F. The attractant and the lights drew the flies to the medium and oviposition was completed in about 4 hours.

To facilitate removal of the egging device from the cage after oviposition was completed and to prevent escape of flies, the cages were moved into a chilling room where the temperature was regulated to 34° to 40° F. When the flies became immobilized the egging device and other contents of the cage were removed. The flies, paper toweling and pupae cases were placed in paper bags and destroyed by heating for 3 days at 140° F. and by fumigation with methyl bromide for another 24 hours and then by incineration. The egging device was carried into the egging room, where the eggs were collected and weighed into 6-gram lots. Each lot was divided equally and the eggs were then placed on moist toweling in paired petri dishes. A small pat, about 1 ounce, of meat was placed in each dish to sustain the young larvae from the time of hatching until they were placed on starting medium. The eggs were held at 80° F. and hatched in 12 to 14 hours.

Larval Rearing.—The newly hatched larvae and the contents of each paired petri dishes were distributed evenly on the starting medium in a galvanized pan 16×26×4 inches. The starting medium was prepared by thoroughly mixing 90 pounds of lean ground horse meat, 4 gallons of a 1:1 water-bovine plasma solution, and 180 cc. (0.2%) of formaldehyde. It was placed evenly about one-half inch deep in starting pans, sufficiently in advance of "seeding" the larvae to allow it to reach a temperature of 98° to 102° F. For 24 to 30 hours the young larvae were maintained in the starting room, 10×100×10 feet, regulated to the same temperature and relative humidity of 85% to 90%. Because the young larvae develop better under constant temperatures they were maintained in a heated room, where the pans were moved manually on a monorail, rather than on vats where there was greater fluctuation of temperatures. The availability of eggs and the desired level of production determined the number of pans seeded with young larvae for each 8-hour shift. During the greater portion of the Sebring, Florida plant operation, 54 pans per shift, or 162 pans per day were used. When there were not enough eggs to meet a shift-production quota, an effort was made to make up the deficiency on the following shift. By following this procedure rarely was the weekly average of egg production below the total fly production requirement. During the 24 to 30 hours the larvae remained in the starting room, all the pans were examined every 15 minutes. Excessive moisture was either sponged off or bits of horse meat were dropped on the surface to aid absorption. If cultures were too dry, the medium was moistened with the plasma-water mixture.

The feeding routine of larvae from the second through the sixth day varied owing to several factors—amount and rate of medium consumption; temperature and humidity of the rearing area; availability of adequate rearing-medium ingredients, particularly blood; and whether [illegible] occurred any power failures or interruptions ow[illegible]o equipment breakdown. Should any of these factors begin to affect larval development, adjustments in procedures must be made immediately if production is to be maintained.

About 2 hours before the larvae were to be transferred from the pans in the starting room to the rearing vats, two aluminum dividers, one on each side the vat were placed about 8 to 10 inches from the edge. The divider was merely a right-angle piece of metal 3 inches wide and 5 feet long cut to fit the vat closely at each end. A piece of thin plastic sheeting 18 inches wide and about 6 feet long was draped over the area framed by the divider, in order to confine the medium along each side of the vat and to keep the blood from oozing over the unused portion of the vat. Into these two areas the rearing medium (horse meat $\frac{3}{16}$ inch grind or whale meat $\frac{1}{8}$ inch grind) was spread uniformly about ½ to ¾ inch deep. When it had warmed to 98° F. the young larvae were brought from the starting room. A 2-inch furrow was made the length of the vat on each side, pushing the medium toward the barrier. Pieces of medium containing larvae were lifted from the pan and placed in the furrow. Contents of one pan were used to infest each vat, and the larvae distributed equally along each side of the vat. Vats were inspected periodically during all shifts, but special effort was made during the first 24 hours to see that the correct temperature and moisture of the medium was maintained. If the larvae had consumed their food, the divider was moved a few inches and a fresh strip of rearing medium was spread in the clear space. Pockets of larvae isolated in spent medium were removed and placed with the main body of larvae.

During the second or third day of larval development the spent medium and barriers were removed and the plastic was carefully pulled from beneath the medium. The medium containing the larvae was moved to the edges of the vat, and the area in the center of the vat was filled with fresh rearing medium. If the larvae did not consume all their food, the center feeding was delayed until the third day of larval development. The center feeding permitted the larvae to feed in any direction, and by this time some of them were mature enough to crawl from the vats. At this time the vats were positioned over the grated floor through which the larvae fell into the collecting funnels. The first larvae reached maturity on the fourth day, a larger number on the fifth day, and by the sixth day most of them had left the vats. Spent medium was removed periodically from the center of the vat to lower the level of the medium and provide space for adding just enough food along the edges for the larvae to complete development.

During the last 60 to 90 days of fly production when fewer flies were required for field release, oat and cotton seed hulls were incorporated as adjuncts to the rearing medium in varying amounts, as suggested by the Methods Development Unit of the screw-worm eradication program. These adjuncts reduced the overall cost of the rearing medium, but resulted in the plugging of vacuum and sewage lines and adverse working conditions for personnel. When lines became plugged the medium had to be

removed by hand and often the lines had to be dismantled resulting in a spillage of larvae on the floor and creating a security problem of considerable magnitude. The intense irritation to personnel necessitated showering several times during their 8-hour-tour of duty.

In an assembly-line production system of this nature, vats had to be removed from over the grated floor area on the sixth day to make room for the 3-day-old larvae ready to be positioned over the collecting funnels under the grate. In order to increase the efficiency of plant production, pockets of larvae remaining in the medium on the sixth day were picked and consolidated in a "hospital row" of vats, where they were given additional food and allowed 24 to 36 hours longer to complete development. It was not considered good procedure to maintain these "hospital vats" longer than 36 hours, because of the possibility of getting disease started in the culture.

It is important to get the spent medium off the rearing vats promptly, as this material is a source of ammonia which is toxic to the larvae and nauseous to plant personnel. The spent medium was vacuumed from the vats by 20-inch vacuum, when routinely feeding the larvae or when the culture had left the medium, through 2-inch flexible hoses connected to tanks outside the plant. When each tank became about half full, the medium was heated to 150° F. for 3 hours under 40 pounds steam to insure that all larvae were killed before it was emptied into the sewage.

Pupae Handling.—When the larvae completed development, they crawled to the edges of the vats and fell through the grate portion of the floor into eight large (6—26×32 and 2—32×32 feet) collecting funnels, 4 in each of 2 rows, tapered to an opening 1 foot square. Under each row of funnels was a belt conveyor 117 feet long and 20 inches wide. The belt moved at the rate of 117 feet every 4 hours. On these conveyor belts were placed galvanized pans 18×26×4 inches to catch the larvae as they dropped through the funnels. Each pan contained 8 liters of dry silica sand of 8 to 20 gauge. These pans were interlocked, and as they passed under the funnels each pan collected from 1 to 5 liters of larvae. The pans were then removed from the conveyor belt, and an attendant examined them to determine that there were not more than 1 to 2 liters of larvae per pan. The pans were then loaded onto carrier racks, which moved along an overhead powered monorail at the rate of 360 feet each 8 hours. During the first 8-hour trip a few larvae pupated, but the most of them did not pupate until the second trip.

After each 8-hour trip on the monorail, the pupae were separated from the sand by a power-driven sifter. This sifter was 15 feet long and 18 inches wide with one end elevated about 5 degrees to cause the pupae and larvae to flow onto a conveyor. As the sand was removed the larvae and pupae were sprinkled evenly onto a ¼-inch-mesh wire conveyor. This conveyor, approximately 50 feet long and 4 feet wide, moved at the rate of 50 feet each 30 minutes. The larvae readily crawled through the wire mesh, leaving the pupae on the conveyor which emptied into a hopper. Once each hour the pupae were measured into 2.3-liter lots and placed in wire trays 18×26×2 inches for aging. Each tray was labeled, showing the date, shift, and hour of pupation, and this information remained with each lot until the flies emerged and the release cartons were loaded in the plane just prior to distribution in the field. The larvae that crawled through the wire-mesh conveyor were again measured into 1.5-liter lots and put into pans with clean sand and again recirculated for 8 hours on the powered monorail. This procedure was repeated every 8 hours until all larvae had pupated. When it became necessary, larvae and pupae sifting could be done every 4 hours. This narrowed the age of pupae in any given lot.

After each hour of sifting, pupae in the hopper were measured into 2.3-liter lots and put in wire trays, labeled and then the trays placed in carrier racks which hold 72 trays. As each rack was filled, it was moved manually on a monorail into the pupae holding room where the pupae aged. This room was maintained at a constant temperature of 80° F. and 70% to 80% relative humidity. The pupae were held in this room for 5½ days ±6 hours, and then delivered to the Radiation Section of the screw-worm eradication program for loading into canisters and ultimate irradiation.

DISCUSSION.—From January 5, 1958, through October 26, 1959, 3,802,395,587 pupae were produced by these procedures for the screw-worm eradication program conducted in the Southeastern States. Of this number, 285,615,587 were produced in the Bithlo, Florida plant used as a pilot plant during the development of the mass-rearing procedures. Production in the Sebring, Florida plant began in July 1958, and the highest single-month's production was 329,160,000 pupae in November of that year. On November 23, 1958 the highest single-day's production, 15,430,000 pupae were harvested. Based upon the sustained production of the Sebring plant for 16 months and since maximum production was never reached, it is reasonable to assume that production in excess of 100 million flies per week might have been possible. A summary tabulation of production for the eradication effort follows:

Plant Location	Eggs Obtained (gm.)	Meat Used (lb.)		No. Pupae Produced
		Horse	Whale	
Bithlo	27,300.3	388,059	39,739	285,615,587
Sebring	413,578.4	1,769,160	4,164,120	3,516,780,000
Total	440,878.7	2,157,219	4,203,859	3,802,395,587

Approximately 1,000 pounds of eggs were obtained for production and based on 20,000 larvae per gram of eggs this was a potential production of 8,817,574,000 flies. Of the total amount of horse meat used 78,000 pounds were utilized for oviposition and as food for the flies in the colony. Some other materials utilized in production were citrated bovine blood, 186,058 gallons; sodium citrate, 8,000 pounds; formaldehyde, 60 drums (55-gal.); and 100 drums (55-gal.) of honey.

Personnel requirements and to some extent the material requirements did not increase or decrease proportionately to the production level. This was owing primarily to automation of several phases of the overall plant operation and the expanse of the plant and diversity of operational procedure. A number of persons were required to operate the plant on a 24-hour day, 7-day-week basis regardless of the production level. Figure 1 shows the number of grams of eggs obtained and the number of grams required to

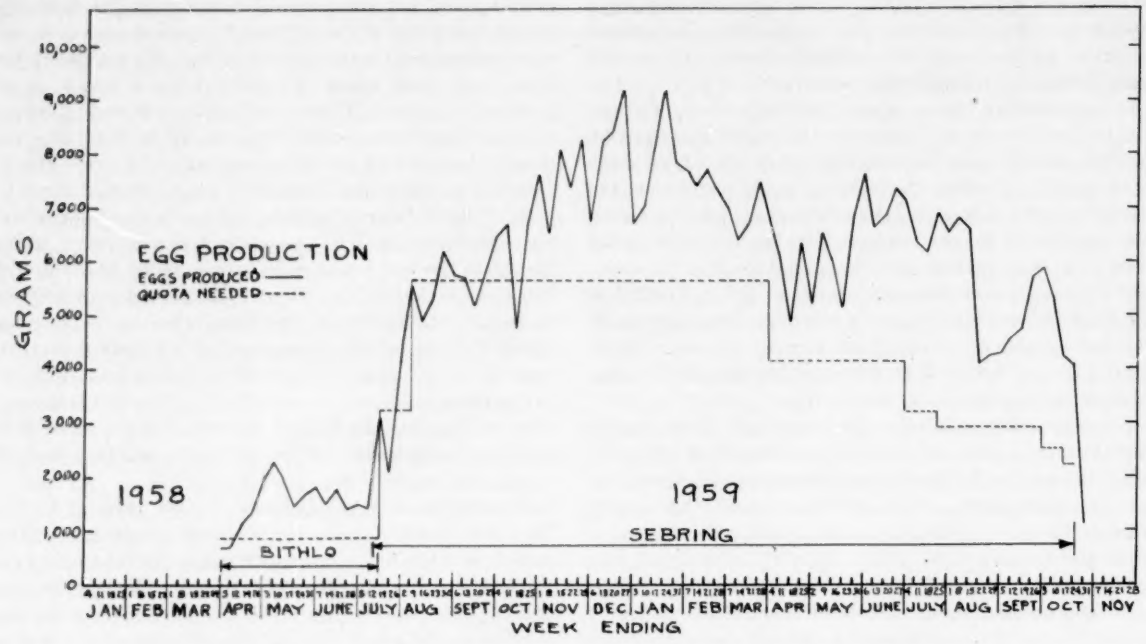

FIG. 1.—Grams of eggs obtained and grams required to meet weekly fly production.

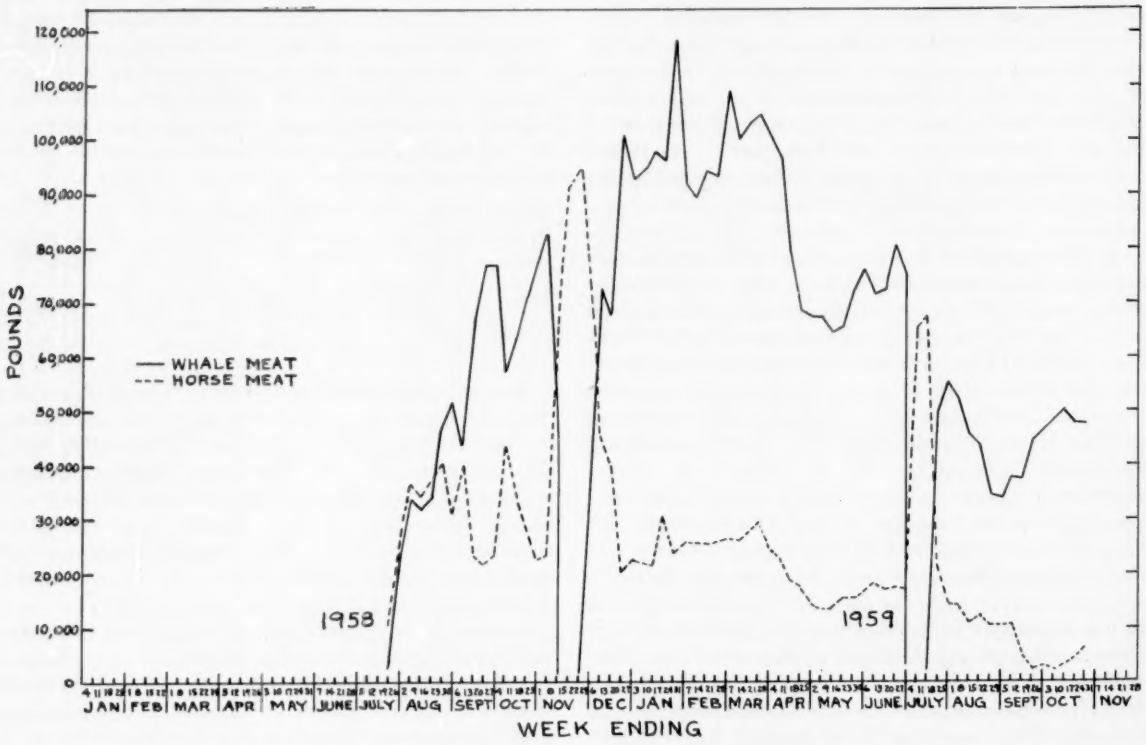
POUNDS
WHALE MEAT
HORSE MEAT
1958
1959
JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV
WEEK ENDING

FIG. 2.—Amount of horse and whale meat used weekly in fly production, Sebring, Fla. 1958–59.

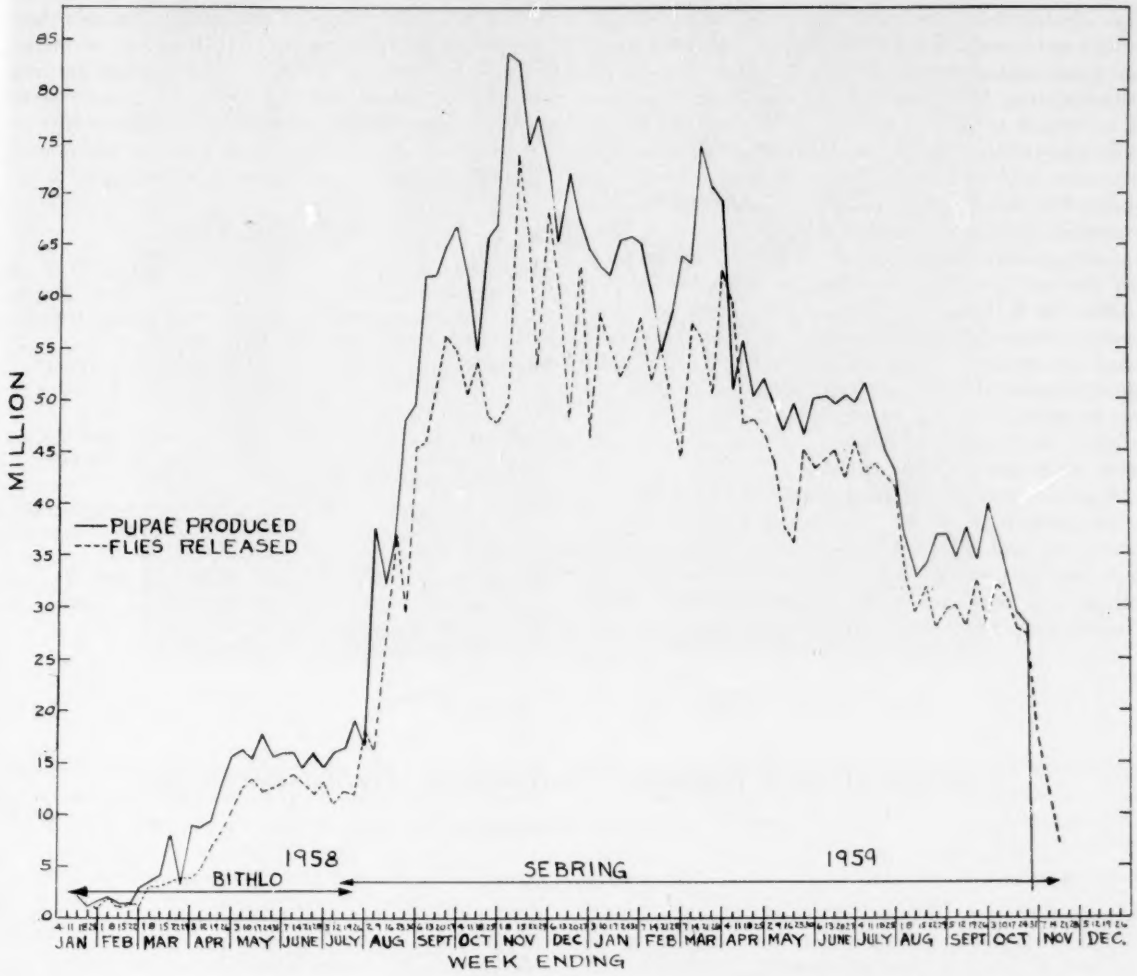

FIG. 3.—Number of pupae produced in relation to number of flies returned.

meet the minimum weekly fly production quota. It is of interest to note that rarely was the weekly quota of eggs required to meet production greater than the available supply of eggs. This occurred usually before the fly colony had been built up to full strength or because of poor emergence of flies. Once the fly colony was built up to full strength and maintenance temperatures and humidities remained adequate, little trouble was experienced in obtaining sufficient eggs for production.

Figure 2 shows the amount of horse and whale meat utilized during the Sebring plant operation. During the Bithlo plant operation the use of whale meat was sporadic as well as experimental and the data are not shown. On two occasions the larvae were maintained entirely on horse meat, because shipments of whale meat were not delivered on schedule. Except for the later part of the eradication program, whale meat ($\frac{3}{4}$-inch grind) was used only during the center feeding and while the larvae were over the grates. During the last 7 or 8 weeks the grind of whale meat was changed to $\frac{3}{8}$-inch grind, and the larvae were placed on the rearing medium made from it immediately out of the starting room. This change is reflected in the low utilization of horse meat during the later part of the operation and the influence of the change in overall cost is readily apparent with the cost of horse meat about 25 cents and whale meat 12 cents per pound.

Figure 3 shows the relation of total number of pupae produced and the number of flies released in the field. Flies released in the field are based on 90% emergence of the number of pupae irradiated and packaged for release. It should be noted that the pupae produced 1 week were released as flies about a week later. Considering this fact and that approximately 5% of the total production was diverted into the stock fly colony for perpetuation, the peaks and dips are quite similar.

MAINTENANCE OF FLY SECURITY.—Fly security, which assures that no viable screw-worm escapes from the production, was of utmost importance and an absolute necessity for the eradication program to succeed. Every effort was made to prevent such an escape. This phase of the operation became more important as the eradication program progressed. By the time production ceased and all pupae had been irradiated, every conceivable means of security except actual use of insecticide such as tanglefoot

strips, electric traps, troughs in front of doors, vacuum cleaners and many other innovations was used within the plant to kill, collect and prevent the escape of viable forms of screw-worms. All objects entering and leaving the plant had to do so in accordance with procedures established by the Security Officer. In general, those objects leaving the plant were held in a room where the temperature was regulated to 140° F. for 48 to 72 hours and then exposed for another 24 hours to methyl bromide fumigation. Objects such as blood cans and meat drums were washed and then steamed or held several minutes in scalding water and then carried immediately to the hot room, where they remained until called for by the meat crew. The cans were passed out through the meat "pass through" and individually inspected by the attendant on duty. Objects too large to enter or leave the plant through the meat "pass through" were handled through the truck security entrance under special treatment.

All persons entering the plant were checked in and out by the guard on duty. They removed their clothing in the locker room and passed down a hallway to the laundry where they were issued clothing. As they left the plant, all clothing was removed in the laundry and they were required to shower, including hair rinse, before they left the security area. Workers and visitors were also checked to see that nothing was removed from the plant unless properly fumigated or treated. Clothing worn by workers and visitors were washed and sterilized within the security area. All persons who entered the fly colony area were given special attention and inspection to make sure no viable flies were brought out into the rearing area.

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Control of the Cranberry Fruitworm, *Acrobasis vaccinii*[1]

William E. Tomlinson, Jr.[2]

Abstract

Though the cranberry fruitworm, *Acrobasis vaccinii* Riley, has been an important cranberry pest since early in the development of cranberry growing, control by flooding was the only reliable control known until 1937 when it was found that rotenone gave effective control. Control methods as recently as 1955 in Massachusetts were based on water management, or application of rotenone or cryolite. Beginning in 1953, tests have been made with many insecticides, applied as dilute high gallonage sprays. Several organic phosphate insecticides and Sevin® (1-naphthyl-methylcarbamate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) as well as rotenone, gave excellent control while cryolite gave erratic results. As semiconcentrated sprays, parathion and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) were superior to Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and methyl parathion, while malathion was unsatisfactory. All phosphates tested exhibited ovicidal action, and several systemics, when applied as concentrate sprays, killed significant numbers of larvae feeding in the fruit.

Very early in the development of cranberry growing on Cape Cod, the cranberry fruitworm (*Acrobasis vaccinii* Riley) was recognized as an important pest of the crop. Eastwood (1859) reported it to be "one of the greatest enemies that the cranberry cultivator has to contend against." White (1870) included a report made by William C. Fish to the Cape Cod Cranberry Growers Association in 1869, which discussed the cranberry fruitworm and its work in considerable detail. Fish also mentioned the only effective fruitworm control measure known until quite recently, that of holding the winter flood water until after mid-May. Figure 1 shows a cranberry opened to expose a cranberry fruitworm larva and the injury it causes.

Franklin (1928) stated that there was no practicable way to treat it on strictly dry bogs. The same author (1937) reported the first effective chemical control of this pest with rotenone, and in 1940 he also reported that cryolite gave effective control. The application of these two insecticides or late holding of the winter flood were the only methods of control recommended in Massachusetts as recently as 1955. Since then parathion dusts and concentrated sprays, applied by aircraft, have become standard treatments, except where this material cannot be used safely.

Plot Tests.—Each year, 1953 through 1959, insecticides and combinations have been tested and compared for effectiveness in controlling cranberry fruitworm. Insecticides were evaluated according to their performance

[1] Contribution No. 1248 of the Massachusetts Agricultural Experiment Station and Entomological Contribution No. 1332 from the Department of Entomology and Plant Pathology of the University of Massachusetts. Accepted for publication June 20, 1960.

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FIG. 1.—Cranberry opened to show cranberry fruitworm larva and injury it causes.

as high gallonage sprays (300 to 400 gallons of finished spray per acre) in square rod plots replicated three times, except in 1955 when plots were not replicated and therefore the data obtained are omitted from this report.

The first spray application was made at the recommended time, *i.e.*, when four or more unhatched, unparasitized eggs per 100 berries were present, and a second application was made 10 days later, or as near that time as weather permitted.

In 1953 and 1954, berries were picked from the centers of the replicates with a cranberry scoop to give a composite sample of at least 1,000 berries per treatment. In 1956, samples were increased to 1,000 berries per replicate, or a total of 3,000 per treatment. Sampling was done by hand picking in 1957, 1958, and 1959, rather than by scooping, to insure the gathering of smaller berries and the shrivelled skins or husks left by the fruitworm, many of which passed through the teeth of the scoop and were therefore not included in the sample. For this reason, the control shown for the years 1953, 1954, and 1956 is probably better than the figures indicate because of the many husks missed in the check plots, while the figures for 1957, 1958, and 1959 are more accurate.

The per cent control was calculated by Abbott's Formula (Abbott 1925) in which $(x-y)/X \times 100$ = per cent control where X = per cent of infested berries in the check plots, y = per cent of infested berries in the treated plots, and $x-y$ = per cent killed by the treatment. The number of berries infested in the check plots varied considerably from year to year, ranging from 5.2% to 24.4%. The per cent reduction in each year is figured with the per cent infestation figure applicable to that year.

As shown in table 1, the organic phosphates, malathion, parathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), averaged more than 90% control of fruitworm when tested two or more seasons, as did the nonphosphate insecticides, rotenone, Sevin® (1-naphthyl methylcarbamate) and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide). Rotenone compared very favorably in effectiveness with the better phosphates, clearly showing why it was such a boon to the cranberry growers when it was first found to be an efficient cranberry fruitworm killer. Cryolite was rather erratic, giving only poor to fair control in 1953, 1956, and 1957 when July rainfall was above average, whereas in 1954 when July was drier than normal, control was excellent. Among the materials tested one year only, Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) 68%–70% and related materials), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) and endrin also reduced fruitworm infestations more than 90%.

Some blasting of open blossoms and dwarfing of developing fruit resulted from applications of malathion, particularly under poor drying conditions, so that it has been recommended only with reservation, even though it has given excellent fruitworm control. Dibrom also caused injury, typically a callousing of the berry surface at the calyx end, but callousing occurred anywhere on the fruit where spray droplets dried slowly.

RATES AND METHODS OF APPLICATION.—In addition to these evaluation studies, some tests of other rates and methods of application were also made. These included

Table 1.—Cranberry fruitworm insecticide evaluation tests, 1953–1959.

Insecticide and Formulation[a]	Active Ingredient in 300 Gal./A. (lb.)	Per Cent Control					
		1953	1954	1956	1957	1958	1959
25% malathion WP	1.875	94.6	98.1	—	—	—	—
57% malathion EC	2.25	97.8	90.4	98.6	—	—	—
15% parathion WP	0.9	—	100.0	96.0	—	—	—
Parathion 4 flowable	0.75	—	—	—	99.7	93.7	100.0
5% rotenone+2 oz. spreader[b]/100	12.0	92.5	—	96.9	87.4	—	—
5% rotenone+DDT WP	6.0	—	—	97.3	—	—	—
+2 oz. spreader/100	3.0						
Cryolite	21.0	11.8	98.1	70.4	69.1	—	50.6
Ryania+2 oz. spreader/100	21.0	50.5	82.7	96.0	—	—	—
Ryania+	10.5						
50% DDT WP +2 oz. spreader/100	3.0	—	95.8	75.9	—	—	—
50% DDT WP	3.0	66.6	—	79.4	—	—	—
25% Diazinon WP	2.25	—	100.0	98.3	94.7	88.9	79.4
50% Dylox®[c] SP	3.0	—	—	—	85.0	94.4	—
50% Sevin WP	3.0	—	—	—	92.4	98.6	99.6
25% ethion WP	2.25	—	—	—	53.8	—	86.1
25% Guthion WP	1.5	—	—	—	98.1	95.1	—
50% Thiodan WP	2.25	—	—	—	—	93.7	96.9
Phosphamidon 4 SL	1.5	—	—	—	—	99.3	79.4–(.75 lbs.)
25% EPN WP	1.5	97.8	90.4	—	—	—	—
50% Methoxychlor WP	3.0	63.4	—	—	—	—	—
50% TDE WP	3.0	65.5	—	—	—	—	—
50% Dieldrin WP	3.0	—	88.5	—	—	—	—
Delnav EC 4 lb./gal.	3.0	—	—	—	—	95.8	—
Dibrom EC 8 lb./gal.	3.0	—	—	—	—	92.4	—
Endrin EC 1.5 lb./gal.	0.6	—	—	—	—	—	99.7
Trithion 8 flowable	1.5	—	—	—	—	—	97.9
46% dimethoate SC	1.5	—	—	—	—	—	89.7
Check (% infested berries)		9.3	5.2	22.3	12.0	19.2	24.4

[a] WP, wettable powder; EC emulsion concentrate; SC, soluble concentrate; SL, soluble liquid; SP, soluble powder.
[b] Triton B—1956. Rohm & Haas Co., Philadelphia, Pa.
[c] *O,O*-Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate.

Table 2.—Concentrate and semi-concentrate spray tests against the cranberry fruitworm.

Insecticide	Type of Applicator	Gallons of Spray per Acre	Active Ingredient per Acre (lb.)	Per Cent Reduction			
				1956	1957	1958	1959
Malathion EC	Helicopter	10	1.14	37.6			
Malathion EC	Hydraulic	100	1.14	64.5			60.2
Malathion EC	Weed sprayer	25	2.25		50.6		
Methyl Parathion EC	Weed sprayer	25	1.0			79.9	
Diazinon EC	Weed sprayer	25	1.0			80.6	
Parathion flowable	Weed sprayer	25	1.0			99.7	97.2
Trithion flowable	Weed sprayer	25	1.0				99.5

Table 3.—Ovicidal action of phosphates against the cranberry fruitworm.

Insecticide	Type of Applicator	Gallons of Spray per Acre	Active Ingredient per Acre (lb.)	Per Cent Kill		
				1956	1958	1959
Malathion EC	Helicopter	10	1.14	33.3		
Malathion WP	Hydraulic	300	1.875	66.6		
Malathion WP	Hydraulic	300	1.5		88.0	
Parathion flowable	Weed sprayer	25	1.0		85.7	
Methyl Parathion EC	Weed sprayer	25	1.0		57.1	
Diazinon EC	Weed sprayer	25	0.5		50.0	
Diazinon WP	Hydraulic	100	1.0		50.0	
Parathion flowable	Sprinkler		0.5			62.5
Parathion flowable	Weed sprayer	25	0.5			50.0
Malathion WP+	Hydraulic	100	1.0			65.0
DDT WP			2.0			

concentrated sprays applied by aircraft where the insecticides were applied in 10 or less gallons of water per acre, as semi-concentrate sprays applied by a low pressure (60 p.s.i.) boom-type weed sprayer with an application rate of 25 gallons per acre, and as high pressure (400 p.s.i.) hydraulic sprays applied at 3X concentrations in 100 gallons of water per acre, rather than at the usual 300 to 400 gallons per acre rate.

Plot sizes were large and varied and not replicated in these tests. Berry samples were scooped or hand picked at random in three or more places within the sprayed plot, and control was determined by comparison with berries picked from nearby, comparable, unsprayed areas. Results are summarized in table 2. Parathion and Trithion as semi-concentrate sprays were very effective. Methyl parathion and Diazinon were moderately effective, while malathion was unsatisfactory as a concentrate 3X spray.

OVICIDAL ACTION.—Observations as to the ovicidal action of some of the phosphate insecticides were made in some of the tests. The number of eggs killed varied but there was some kill whether they were applied as concentrate, semi-concentrate or high-gallonage sprays. Table 3 summarizes the observations on ovicidal action in tests with malathion, methyl parathion, parathion, Diazinon and Trithion.

SYSTEMIC ACTION.—Also tested in a limited way were several systemic insecticides and parathion. Results were evaluated by counts of killed and surviving larvae in infested fruit from systemic treated plots. Results summarized in table 4 show that when applied as concentrates Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), phosphamidon, dimethoate and parathion killed a large number of the larvae feeding in the fruit. When applied as semi-concentrates they were very much less effective.

PRESENT CONTROL PRACTICES.—The reason for the adoption by the cranberry growers of aircraft-applied parathion dusts and concentrated sprays for fruitworm control since 1955 is apparent because of its overall effectiveness in controlling the larvae, even where they are feeding within the berry itself. Because of its effectiveness and economy, parathion concentrated sprays at the present time have almost completely replaced other materials and methods for fruitworm control, except in proximity to built-up areas where less hazardous insecticides are recommended and used.

Table 4.—Larvicidal action of systemic insecticides against the cranberry fruitworm.

INSECTICIDE	GALLONS OF SPRAY PER ACRE	ACTIVE INGREDIENT PER ACRE (LB.)	PER CENT KILL 1956	PER CENT KILL 1959
Phosdrin Tech.	8	0.5	83.7	
Phosdrin Tech.	8	1.0	63.0	
Phosphamidon 4 Sol. Liquid	5	0.5		84.1[a]
Phosphamidon 4 Sol. Liquid	5	1.0		82.5[b]
Phosphamidon 4 Sol. Liquid Weed Sprayer	10	0.5		42.1
Phosphamidon 4 Sol. Liquid Weed Sprayer	25	0.5		28.6
Dimethoate EC	5	0.5		61.3
	5	1.0		58.3
	100	0.5		0.0
Parathion Flowable	5	0.5		66.6
	100	0.5		34.3

[a] Average of three tests.
[b] Average of two tests.

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Biology and Control of the Morning-Glory Leaf Miner, *Bedellia somnulentella*, on Sweet Potatoes[1]

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ABSTRACT

Larvae of the morning-glory leaf miner, *Bedellia somnulentella* (Zell.), caused extensive damage to the foliage of sweet potatoes in San Diego County, California, during 1959. Biological studies of this leaf miner revealed that there are five larval instars. First- and second-instar larvae form a serpentine mine; larvae in the last three instars form blotch mines, each larva constructing and abandoning several blotch mines during the course of its development. Naturally occurring populations of larvae are heavily parasitized by *Apanteles bedelliae* Viereck. Chemical evaluation studies showed that the larvae can be controlled by the use of toxaphene, Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), parathion, malathion, DDT, or Diazinon® [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate].

The unusual appearance and bionomics of larvae of the morning-glory leaf miner, *Bedellia somnulentella* (Zell.) (Lepidoptera: Lyonetiidae), were first described in American literature by Clemens (1862). Although the insect

[1] Paper No. 1228, University of California Citrus Experiment Station, Riverside, California. Accepted for publication June 20, 1960.

[2] The authors gratefully acknowledge the assistance of R. L. Hale and H. Nakakihara, University of California Citrus Experiment Station, Riverside, who aided in the control investigations. Parasites were reared by E. I. Schlinger, University of California Citrus Experiment Station, Riverside, and identified by C. F. W. Muesebeck, U. S. National Museum. Specimens of the morning-glory leaf miner were identified by G. T. Okumura, California State Bureau of Entomology, and by H. T. Capps, U. S. National Museum. Insect photographs were taken by L. R. Brown, Department of Entomology, University of California, Los Angeles, and A. S. Deal, Entomologist, University of California Agricultural Extension Service.

FIG. 1.—Damage caused to sweet potato foliage by larvae of the morning-glory leaf miner.

infests a variety of plant species belonging to the family Convolvulaceae, no report of it as an economic pest of any crop has appeared in the literature. Needham *et al.* (1928) report that morning-glory, *Ipomoea purpurea* (L.), is the preferred host. The leaf miner was considered by Smith (1950) to be the most important insect attacking the foliage of bindweed, *Convolvulus* spp., in Kansas.

During July, 1959, the morning-glory leaf miner was observed causing extensive damage to the foliage of sweet potatoes, *Ipomoea batatas* L., in San Luis Rey, San Diego County, California. Subsequent surveys revealed that infestations were present in San Diego and Orange Counties on sweet potatoes and on various wild host species belonging to the genus *Convolvulus*.

DAMAGE.—In sweet potato fields having a heavy density of the morning-glory leaf miner, many larvae were found within the confines of each leaf. Under these conditions the mines soon became confluent, with most of the leaf material except the upper and lower epidermises being consumed (fig. 1). The mined leaves became brown and shriveled but remained on the stems, imparting a heavily frosted appearance to the plants. Population densities within the San Luis Rey area were so high that most plants probably would have lost the major portion of their photosynthesizing surface if insecticide treatments had not been made.

Only sweet potato fields that were within a 5-mile radius of San Luis Rey were damaged to the degree noted above. Low levels of infestation or signs of a previous infestation, often exemplified by the presence of parasite cocoons and empty mines, were found in many other fields. The factors responsible for a high population density in one area but not in others have not been determined.

GROSS APPEARANCE OF STAGES.—Clemens (1862) briefly described the mature larvae, the pupae, and the adults of the morning-glory leaf miner; Forbes (1923) recorded distinguishing characteristics of the adults. Additional observations of gross appearance of the various stages were made during July, August, and September, 1959.

The eggs are translucent white, appearing as hemispheroids flattened against the leaf surface. The chorion is characterized by a granulate appearance. Approximately 1 day before hatching, the tan larval head capsule can be seen within the egg. Lengths and widths of eggs were measured with the aid of a binocular microscope; these data are summarized in table 1.

Table 1.—Measurements of eggs and larval head capsules of the morning-glory leaf miner.

STAGE OF DEVELOPMENT	SIZE IN MILLIMETERS ±95% Confidence Mean	Interval	RANGE	NUMBER EXAMINED
Egg				
Length	0.335	0.010	0.30–0.39	24
Width	.230	.007	0.17–0.25	24
Larva (head capsule width *Instar*				
First	0.098	0.003	0.09–0.11	24
Second	.155	.004	0.15–0.17	18
Third	.221	.003	0.21–0.23	16
Fourth	.310	.011	0.27–0.34	18
Fifth	.453	.008	0.39–0.49	35

The basic body color of larvae of each of the five instars is light yellow-grey; the gut is evident as a bright green mid-line except immediately before a molt, when the intestinal contents are voided. The prognathous head is retractile into the prothorax and is alternately advanced and withdrawn during feeding. First-instar larvae appear to be legless; thoracic legs and prolegs appear in the second instar and are present thereafter. During the third instar, pairs of faint pink spots appear dorso-laterally on thoracic segment 3 and abdominal segments 2, 3, 6, 7, and 8. In the fourth instar, the spots disappear, and dark-red tubercles appear laterally on all thoracic and abdominal segments. Smaller, white tubercles, in addition to the red tubercles, are found on the fifth-instar larva (fig. 2).

During a brief prepupal stage the basic body color changes to light green and the tubercles become less pronounced. Newly formed pupae are light green with a mottled red pattern appearing in places corresponding to the location of the larval tubercles. The red color gradually diminishes and the mature pupae vary in color from light green to dark brown.

FIG. 2.—Fifth-instar larva of the morning-glory leaf miner.

The adult is a small, tan-to-grey moth, the sexes of which are easily distinguished. The male has a crown of hairs surrounding the disc-shaped genital claspers; these structures are lacking in the female.

BIOLOGY.—Clemens (1862) reported that the early larval mine of the morning-glory leaf miner is narrow, very serpentine, and contains a central line of frass. The larva soon leaves its serpentine mine and thereafter mines the leaf in blotches, entering between the cuticles from the lower surface. The entrance to the blotch mine is kept open, and all frass is voided externally. Numerous cross-threads are constructed below the leaf, around mined areas; these are freely traversed by the larva in moving from one place to another. The naked pupa is fixed by its posterior extremity to a junction of cross-threads and is supported in a horizontal position by other threads.

Additional biological studies were conducted in the field and the greenhouse during 1959 and 1960. The greenhouse was maintained at a temperature of approximately 80° F. and a relative humidity of approximately 50%. Eggs, larvae, and pupae of known age were obtained by placing potted sweet potato plants in an oviposition cage containing morning-glory leaf miner adults for a 24-hour period.

As far as could be determined, adults in the presence of sweet potato plants laid all their eggs on the leaves, with 67% of 165 eggs counted being deposited on the lower epidermis. There was no concentration of eggs toward the center or periphery of each leaf; however, most eggs (94%) were laid adjacent to veins. The majority of eggs was laid singly, though on occasion two or more eggs were found adjacent to each other.

Upon hatching, the larva penetrates through the portion of the egg adjacent to the leaf epidermis, feeding directly into the leaf and leaving the interior of the empty egg littered with black fecal pellets.

Head capsule measurements at the widest point were made at 2-day intervals on individual larvae of known age taken from potted plants in the greenhouse (table 1). The aggregation of head capsule measurements into five distinct groupings and a specific larval appearance associated with each grouping indicate that there are five instars.

First- and second-instar larvae form a serpentine mine. Excrement is usually left as a continuous chain of black pellets adhering to the upper-epidermis side of the mine. Almost all larvae observed were oriented with their ventral surface toward the upper epidermis.

The second-instar larvae molt at the end of their serpentine mines; third-instar larvae leave the mines, usually making the exit holes in the lower epidermis. The larva moves back and forth over the leaf surface, constructing a loose webbing of silken strands extending to the petiole and other portions of the same and adjacent leaves. Working from this webbing, it cuts a hole in the epidermis and feeds its way into the leaf. Third-, fourth-, and fifth-instar larvae construct only blotch mines. Except during molting, when the larva is entirely within the mine, the terminal portion of the abdomen is always found at, or protruding from, the entrance hole. As a result, before the mine becomes larger in radius than the length of the larval body, the larva leaves, constructs more webbing, and forms a new mine on the same or on a different leaf. Thus, each larva occupies one serpentine and several blotch mines during the course of its development. The excrement, which is voided through the hole in the epidermis, usually hangs down in a continuous webbed chain. As a result, the webbing and lower leaves of heavily infested plants acquire a black-speckled appearance.

FIG. 3.—Larvae of the morning-glory leaf miner in various stages of mining activity and cocoon of *Apanteles bedelliae* Viereck.

Total development time from egg to adult for 153 individuals at a temperature of 80° F. was 20.2 ± 0.2 days. The egg and pupal stages each required approximately 4.5 days and the larval stage 11 days.

In coastal areas of San Diego County, development proceeded on wild host plants during the winter of 1959–60. Despite some dieback owing to frost, these plants continued to grow throughout the winter. Mines containing active larvae were found during inspections made in December, 1959, and February and April, 1960.

PARASITES.—Parasites reared from a series of morning-glory leaf miner larvae collected in the field on July 24, 1959, were all identified as *Apanteles bedelliae* Viereck. The parasite was present in great abundance, as evidenced by a profusion of white cocoons on the lower surfaces of leaves in heavily infested areas (fig. 3).

CONTROL STUDIES.—On July 27, 1959, a number of insecticides was applied to the foliage of nearly mature sweet potatoes in San Luis Rey. Treatments were arranged in a randomized block design with two replicates. Each plot was 3 rows (9 feet) by 35 feet. All insecticides were formulated as sprays and were applied with a 3-gallon hand sprayer through two No. 4 hollow-cone nozzles at a pressure of 40 p.s.i. and a volume of approximately 50 gallons per acre. Twenty-five leaves were sampled at random from the center row of each plot on July 29 and were examined on July 30 under a binocular microscope for the presence of living larvae.

On August 14, 1959, a second test was applied in an adjacent field. Treatments were arranged in a randomized complete block design with four replicates. Each plot was 9 by 40 feet. Sprays were applied with a tractor-drawn Hardy sprayer, using three No. 4 nozzles per row, a pressure of 150 p.s.i., and a volume of approximately 50 gallons per acre. Dusts were applied with a hand duster at a rate of 18 pounds of formulated material per acre. On

Table 2.—Evaluation of insecticides applied to sweet potato foliage for control of larvae of the morning-glory leaf miner. San Luis Rey, California. 1959.

Material	Formulation	Actual Toxicant/A.	Mean Number Miners per 100 Leaves at 3 Days	7 Days
Test 1—July 27				
Toxaphene	Emulsion concentrate	4.3	6[a]	
Dylox	Soluble powder	0.8	3[a]	
Parathion	Emulsion concentrate	0.2	1[a]	
Malathion	Emulsion concentrate	2.1	0[a]	
DDT	Emulsion concentrate	1.5	3[a]	
Diazinon	Emulsion concentrate	0.8	0[a]	
Untreated			78[b]	
Test 2—August 14				
Malathion	Emulsion concentrate	0.7	2[c]	44[f]
	Emulsion concentrate	0.2	22[cd]	92[fg]
Dylox	Soluble powder	0.6	29[cd]	97[fg]
	Soluble powder	0.2	55[de]	33[f]
DDT	Emulsion concentrate	0.7	23[cd]	153[fg]
	Emulsion concentrate	0.2	95[de]	239[g]
	2% dust	0.4	132[de]	155[fg]
Untreated			207[e]	251[g]

[a,b,c,d,e,f,g] Any two means having the same superscript letter are not significantly different according to Duncan's multiple range test at the 5% level. Original data were transformed to logarithms for statistical analysis.

August 17 and 21, 1959, 30 and 21 leaves, respectively, were sampled at random from the center row of each plot. Leaves were examined directly in the field, only those live larvae in blotch mines being recorded.

Application and sampling data for both control tests are included in table 2. Toxaphene at 4.3 pounds, Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) at 0.6 pound, parathion at 0.2 pound, malathion at 0.2 pound, DDT at 0.7 pound, and Diazinon® [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] at 0.8 pound of actual toxicant per acre initially produced effective control when applied as sprays. However, all plots in both tests rapidly became reinfested. This may largely be attributable to migration from the remainder of the fields, which were untreated, and to the short life cycle of the miner. When a single, 3-acre field was treated with 2% Diazinon dust at 1 pound of actual toxicant per acre, larval control was practically complete and no substantial reinfestation occurred during the remainder of the growing season.

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On the Stability of Insecticide-Resistance in Mosquitoes[1]

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Abstract

Laboratory tests on the stability of insecticide resistance in mosquito larvae indicate that some colonies experience a reduction from their initial level of resistance to both chlorinated hydrocarbons and organophosphorus compounds when selection pressure is not maintained. The rapid decline in resistance to organophosphorus insecticides parallels observations made on laboratory colonies of the adult house fly, *Musca domestica* L.

Laboratory tests conducted on a malathion-resistant strain of *Culex tarsalis* Coq. and a parathion-resistant strain of *Aedes nigromaculis* (Ludlow) indicated that resistance levels in field populations of both strains has declined from the previous year.

Field tests in 1958 on the parathion-resistant strain of *A. nigromaculis* indicated that formulation difficulties were not responsible for the number of surviving larvae observed after application of 0.1 lb./acre of parathion. These findings substantiate earlier laboratory tests reported elsewhere that physiological resistance to parathion existed in this strain.

Field tests conducted in 1959 indicated a lower level of resistance to malathion and parathion than for 1958. Fewer treatments and, in the case of parathion-resistance, the substitution of a different insecticide may have been responsible for the decline in resistance.

Rotation of organophosphorous insecticides where cross tolerances do not exist, as well as compounds with differing modes of action, are suggested as a possible means of averting the development of resistance. Prolonged and frequent use of any one type of insecticide on house flies or mosquitoes should be discouraged.

The widespread use in California of organophosphorous insecticides in the control of mosquitoes resistant to chlorinated hydrocarbons has resulted in the development of strains resistant to both types of compounds (Gjullin & Isaak 1957, Lewallen & Nicholson 1959).

The stability of insecticide-resistance in mosquitoes is of particular interest when comparisons are made with results obtained on the house fly, *Musca domestica* L., since control measures against both types of insects frequently employ the same insecticides.

Stability of Chlorinated Hydrocarbon Resistance.—Although considerable information is available on the stability of chlorinated hydrocarbon resistance in the house fly, little seems to have been done in California to determine the stability of chlorinated hydrocarbon resistance in mosquitoes. Isaak (1956) in laboratory tests found that DDT-resistant *Culex tarsalis* Coquillett larvae from Kern County were as resistant to DDT as originally

[1] Accepted for publication June 20, 1960.

[2] The author wishes to acknowledge the able assistance of Mrs. Patricia Gillies in the laboratory, and Mr. James H. Bray in the field. This study was supported in part by funds from National Institutes of Health Grant No. E2875.

reported, even after 4 years of organophosphorous substitution in the field.

March (1959) reported on the stability of resistance to DDT and lindane in the multiresistant field-collected Pollard strain of house fly reared in the laboratory without exposure to insecticides. Resistance to DDT declined very slowly during a 4-year period, reached a level of intermediate resistance, and has remained at this level for an additional 4 years. House fly resistance to lindane, which was initially at a lower level than DDT resistance, has remained about the same during the 8-year period.

The results of tests conducted on a laboratory colony of *Culex pipiens quinquefasciatus* Say obtained from the Orange County Mosquito Abatement District indicated a moderate initial level of resistance to DDT. In comparison with a susceptible laboratory strain the LC_{50} was 3.3 p.p.m. (165×) and the LC_{90} was 6.0 p.p.m. (100×) on July 17, 1959. This colony was maintained without selection pressure and checked on January 4, 1960. At this time the LC_{50} was 0.29 p.p.m. (14.5×) and the LC_{90} 0.8 p.p.m. (14×). In $5\frac{1}{2}$ months (approximately 8 to 10 generations), DDT resistance declined from 165× to 14.5× at the LC_{50} level and from 100× to 14× at the LC_{90} level.

The decline of DDT resistance in a laboratory colony of *C. p. quinquefasciatus* appears to be much more rapid than the cases cited with field-collected *C. tarsalis* and laboratory maintained *M. domestica*. Similarly, a DDT-resistant strain of *Aedes aegypti* (L.) from Trinidad was found to lose a considerable part of its resistance in a year of laboratory culture at Savannah, Georgia (Brown 1958). *Anopheles quadrimaculatus* Say larvae collected at Beaver Dam Creek, Kentucky, and maintained as a laboratory culture were selected with DDT pressure at the LC_{90} level for five generations. The resistance to DDT increased only 3-fold and reverted on relaxation of pressure (Brown 1958). DDT pressure on laboratory reared *Anopheles stephensi* Liston larvae or adults resulted in a 2- to 5-fold tolerance which reverted rapidly on cessation of selection (Davidson 1958).

In general it appears that DDT resistance has a tendency to decline fairly rapidly in laboratory cultures of mosquitoes if pressure is not maintained.

The 100- to 165-fold resistance to DDT in the *C. p. quinquefasciatus* strain from Orange County can be classed as moderately resistant. Higher levels of DDT resistance have been noted in another species of mosquito reared in the laboratory. *C. tarsalis* from Oregon have been reported by Eddy *et al.* (1958) to have an LC_{50} level 1,800× normal and an LC_{90} of 5,000× normal. The magnitude of resistance in this case is comparable to that reported in the house fly.

STABILITY OF ORGANOPHOSPHOROUS RESISTANCE.—According to March (1959) when selection pressure is relaxed with organophosphorous compounds on laboratory colonies of house flies, resistance levels decline very rapidly, becoming clearly evident in from five to seven generations.

The malathion-resistant strain of *C. tarsalis* reported by Gjullin & Isaak (1957) was colonized and tested against malathion on October 15, 1958. These tests indicated an LC_{50} of 0.2 p.p.m. (12×) and an LC_{90} of 1.0 p.p.m. (37×). After 5 months of rearing without selection pressure (8 to 10 generations) a second test with malathion was performed on March 24, 1959. This test indicated that the colony had reverted to the same level as the normal susceptible strain. Unfortunately we do not know how much earlier this reversion to susceptibility had occurred. As was the case with laboratory strains of organophosphorous-resistant house flies, a laboratory colony of an organophosphorous resistant strain of mosquitoes reverted to susceptibility very rapidly.

Table 1.—Response of fourth instar *Culex tarsalis* larvae to malathion.[a]

DATE 1959	LC_{50} P.P.M.	RESISTANCE LEVEL[b]	LC_{90} P.P.M.	RESISTANCE LEVEL[b]
3/24	0.008	No resistance	0.027	No resistance
4/15	.0049	No resistance	.03	No resistance
5/25	.0043	No resistance	.023	No resistance
6/12	.032	(2×)	.08	(2.7×)
7/16	.015	No resistance	.14	(5×)
8/18	.015	No resistance	.054	(2×)
9/15	.013	No resistance	.1	(3×)

[a] Larvae reared in the laboratory from field-collected eggs.
[b] Compared with a susceptible laboratory strain of *C. tarsalis*. LC_{50} 0.016 p.p.m., LC_{90} 0.029 p.p.m.

MALATHION-RESISTANT *CULEX TARSALIS*.—Throughout most of the breeding season of *C. tarsalis* during 1959 a study was made on the response to malathion of laboratory-reared larvae obtained from egg rafts collected at a pasture near Fresno, California, where resistance was known to exist. The testing method employed was identical to that described for *Aedes nigromaculis* larvae (Lewallen & Nicholson 1959). The results are presented in table 1. Despite continued treatment during 1959 with malathion, *C. tarsalis* collected from the pasture where fairly high levels of resistance had existed in 1956, 1957, and 1958 had declined to a low level of resistance in 1959 as shown in table 1. Tests with DDT against this strain indicated that the response was comparable to the susceptible strain. Initial use of malathion in the field against this strain was prompted by the development of chlorinated hydrocarbon resistance. Substitution of an organophosphorous compound in the control program failed to keep DDT resistance at a high level.

PARATHION-RESISTANT *AEDES NIGROMACULIS*.—During 1958, parathion resistance was detected by Lewallen & Nicholson (1959) in *Aedes nigromaculis* larvae from the Kings Mosquito Abatement District (designated as the Hanford strain). When compared with a susceptible strain the initial levels of resistance were 2× at the LC_{50} level (0.00008 p.p.m.) and 68× at the LC_{90} level (0.0068 p.p.m.). Tests with parathion were conducted throughout the breeding season on laboratory reared larvae obtained and treated by the method described by Lewallen & Nicholson (1959). The results of these tests are presented in table 2.

Laboratory tests indicated that parathion resistance had declined from the original 1958 level of 0.0068 p.p.m. (LC_{90} comparison) although chemical treatment was being administered to field populations. Insecticide applications were less frequent during 1959 than in 1958. In addition, Metacide® (60% methyl parathion, 15%

Table 2.—Response of laboratory-reared fourth instar *Aedes nigromaculis* to parathion.[a]

DATE 1959	LC_{50} P.P.M.	RESISTANCE LEVEL[b]	LC_{90} P.P.M.	RESISTANCE LEVEL[b]
3/14	0.00033	10×	0.0011	11×
4/17	.00008	2×	.00016	1.6×
5/ 6	.00095	26×	.0024	24×
7/13	.00021	6×	.0011	11×
9/16	.00025	7×	.0013	13×

[a] Larvae hatched from eggs laid by field-collected females.
[b] Compared with susceptible Pinedale strain. LC_{50} 0.000035 p.p.m., LC_{90} 0.0001 p.p.m.

ethyl parathion) was substituted for parathion in control operations during 1959. It is a matter of interest that the decline in parathion resistance may have been caused in part by the fact that several populations escaped treatment during the season and a different organophosphorous insecticide was substituted in the control program. The highest degree of resistance noted in the laboratory (excluding 1958 tests) was recorded on May 6, 1959 with both the LC_{50} and LC_{90} levels of about the same order of magnitude, 26× and 24×, respectively.

FIELD STUDIES ON PARATHION RESISTANCE.—The dosage rate of parathion recommended for susceptible *A. nigromaculis* larvae is 0.1 lb./acre. Complete mortality in 24 hours can ordinarily be expected with this material when applied as an emulsion concentrate diluted with water.

To eliminate the possibility of obtaining misleading results owing to faulty formulations, controls were run on a susceptible strain at Kerman for comparison with the Hanford strain. Each series of tests for each location was performed with the same manufacturer's formulation. Soil type, vegetation, and depth of water were essentially the same for both localities. The methods employed were consistent with those previously utilized in conducting field tests on new larvicides (Lewallen 1958). Results of the 1958 tests are presented in table 3.

From these results it is evident that considerable numbers of larvae at Hanford survived the dosage of parathion ordinarily used for control. Formulation difficulties were not responsible for these differences since the Kerman strain was controlled at this dosage. One test (not shown in table 3) was performed at 0.2 lb./acre with 99.0% mortality in the Hanford strain. These findings lend further support to the conclusion that a fairly high degree of parathion resistance existed in this strain during 1958. Field tests conducted on the Hanford strain during 1959 are reported in table 3.

DISCUSSION.—The results of both the field and laboratory tests indicate that a lower level of parathion resistance existed in the Hanford strain in 1959 than in 1958. From the evidence gathered in this study it is apparent that organophosphorous resistance in mosquitoes is not stable, and reversion to susceptibility may occur rapidly when selection pressure is relaxed or removed.

Table 3.—Twenty-four hour per cent mortality of *Aedes nigromaculis* fourth instar larvae to field applications of 0.1 lb./acre parathion spray, 1958 and 1959.

DATE	HANFORD STRAIN	KERMAN STRAIN
8/18/58	98.9, 98.5, 99.4	100.0, 100.0
8/19/58	85.9	100.0
8/25/58	100.0	100.0
9/22/58	71.0	
3/16/59	94.0, 98.0, 100.0	
4/14/59	100.0, 100.0, 100.0	
5/11/59	100.0, 100.0, 100.0	
7/22/59	99.0, 99.0	

The genetic basis for the return to susceptibility in resistant insect strains has been discussed by Crow (1957) and Milani (1958).

Some practical considerations of reversion to susceptibility in resistant strains may result in the extended use of organophosphorous compounds through a rotational program with other types of insecticides possessing different modes of action. In addition, the rotation of other organophosphorous insecticides may be employed where cross-resistance does not exist. To avoid the development of resistance it appears that the prolonged and frequent use of any one insecticide should be discouraged, especially on insects with a high biotic potential such as flies and mosquitoes.

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Preliminary Evaluation of Animal Systemic Insecticides[1]

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Abstract

When 17 compounds were found to be active as systemic insecticides in preliminary laboratory tests with guinea pigs, tests were conducted by treating small numbers of cattle with these compounds and Bayer 23129 (*O,O*-dimethyl *S*-2(ethylthio) ethyl phosphorodithioate) to determine their systemic effect against cattle grubs (*Hypoderma* spp.). Bayer 29493, Dowco 105, and Stauffer R-2371 gave 95% to 100% control of grubs at dosages tolerated by the cattle. The rest were ineffective or inconsistently effective or effective at dosages toxic to cattle.

The chemical names of the effective compounds are Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate), Dowco 105 (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ethylphosphoramidothioate), and Stauffer R-2371 (*O,O*-diethyl bis-(4-chlorophenyl) methyl phosphorodithioate).

At the Kerrville, Texas, laboratory, compounds are tested as animal systemic insecticides by a standard screening procedure (Drummond 1958). The compounds that show promise are administered to small numbers of cattle at the laboratory to determine their systemic effect on cattle grubs (*Hypoderma lineatum* (De Vill.) and *bovis* (L.)). This paper presents the results of screening tests with 17 compounds and subsequent tests with 18 compounds against cattle grubs.

The following compounds were used in these tests:

Am. Cyanamid 18706 (*O,O*-dimethyl *S*-(N-ethylcarbamoylmethyl) phosphorodithioate)
Bayer 23129 (*O,O*-dimethyl *S*-2(ethylthio) ethyl phosphorodithioate)
Bayer 24498 (*O,O*-dimethyl *S*-2-(methylsulfinyl)ethyl phosphorothioate)
Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate)
Bayer 25142 (*O,O*-diisopropyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate)
Bayer 25198 (*O,O*-dimethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate)
Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate)
butonate (dimethyl 1-butyryloxy-2,2,2-trichloroethyl phosphonate)
Dition (*O,O*-diethyl *O*-(3,4-tetramethylene umbelliferone) phosphorothioate)
Dowco 105 (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ethylphosphoramidothioate)
General Chemical 4072 (diethyl 2-chloro-1-(2,4-dichlorophenyl) vinyl phosphate)
Monsanto CP-10502 (vinyl dimethyl 1-(dimethoxyphosphenyl) phosphate)
Monsanto CP-10516 (vinyl dimethyl 1-(diethoxyphosphenyl) phosphate)
phosphamidon (1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate)
Shell SD-3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate)
Stauffer R-2371 (*O,O*-diethyl bis(4-chlorophenyl) methyl phosphorodithioate)
Stauffer R-2448 (*O,O*-dimethyl bis(4-chlorophenyl) methyl phosphorodithioate)
Virginia-Carolina 1–13 (*O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate).

The systemic effectiveness of American Cyanamid 18706 has been reported by Hewitt *et al.* (1958) and that of Shell SD-3562, Bayer 25198, Bayer 29493, and Bayer 24498 by Brady *et al.* (1960).

Screening Tests.—Guinea pigs are infested with nymphal lone star ticks (*Amblyomma americanum* (L.)) 2 days and with newly hatched screw-worms (*Callitroga hominivorax* (Cqrl.)) 1 day before treatment. The compounds are formulated as 5% solutions in Tween-20 (polyoxyethylene sorbitan monolaurate) and administered orally or subcutaneously to guinea pigs. Stable flies (*Stomoxys calcitrans* (L.)) are fed on the guinea pigs 4 and 24 hours after treatment and held for 24 hours to determine their mortality. Ticks are checked for engorgement and are held to see if they molt normally. If the guinea pigs or arthropods are killed at the initial dosage of 100 mg./kg., lower dosages are administered until the compounds are no longer lethal.

The results of tests with 17 compounds are summarized in table 1. The results with Bayer 23129 have been reported previously (Drummond 1958).

All the compounds were active as systemic insecticides. In general, screw-worms were most sensitive to the compounds, then stable flies, and finally lone star ticks. This can be explained in part by the relationship of the parasites to the tissues of the host. Screw-worms in wounds are exposed continuously to the insecticides in the blood, other fluids, and tissues, and are therefore subject to their maximum effects. On the other hand, stable flies engorge with blood in a few minutes and effects depend solely upon the amount of insecticide present at the time of engorgement. Ticks are allowed to attach 2 days prior to administration of the insecticide and to feed for 4 additional days, but they are very slow feeders and ingest only small amounts of blood containing various concentrations of the insecticide and its metabolites.

Tests Against Cattle Grubs in Cattle.—Before a compound is administered to cattle, it is referred to cooperating veterinarians[2] for preliminary toxicological studies. With these data available, it is administered to one to four calves at the highest nonlethal dosage. In initial tests a compound is given orally in capsules or as drenches, but often subsequent treatments are administered intramuscularly or dermally. The veterinarians observe the animals for poisoning and determine the effect on blood cholinesterase levels.

Two groups of cattle are available for these tests. One group of calves and yearlings comes from pastures at Camp Stanley, Texas, where the heel fly season (adults of *Hypoderma*) lasts from January to March and grubs (*lineatum*) appear in the animals' backs in September. These animals are usually treated in July. The second group consists of calves imported from the northern United States. The cattle grub-heel fly life cycle is 2 to 4 months later than in southern cattle, and grubs (both

[1] Accepted for publication June 20, 1960.

[2] R. D. Radeleff and assistants, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S.D.A.

Table 1.—Effectiveness of 17 compounds in screening tests with guinea pigs.

Compound	Method of Administration	Lowest Dosage (mg./kg.) Lethal to Guinea Pig	Causing 100% Kill of Screw-worms	Stable Flies	Ticks
Am. Cyanamid 18706	Oral	>100	10	50	50
	Subcutaneous	>100	10	50	100
Bayer 24498	Oral	100	5	25	25
	Subcutaneous	50	5	25	25
Bayer 25141	Oral	2.5	—[a]	—	—
	Subcutaneous	5	1	—	—
Bayer 25142	Oral	5	—	—	—
	Subcutaneous	10	2.5	—	—
Bayer 25198	Oral	>100	5	25	10
	Subcutaneous	100	2.5	25	25
Bayer 29493	Oral	>100	25	25	50
	Subcutaneous	>100	25	*[b]	100
Butonate	Oral	>100	25	100	100
	Subcutaneous	50	—	—	—
Dition	Oral	50	10	—	—
	Subcutaneous	25	—	—	—
Dowco 105	Oral	>100	50	25	No test
	Subcutaneous	>100	50	25	No test
General Chemicals 4072	Oral	>100	*	*	*
	Subcutaneous	50	10	10	—
Monsanto CP-10502	Oral	>100	10	10	50
	Subcutaneous	100	10	25	25
Monsanto CP-10516	Oral	>100	10	25	50
	Subcutaneous	100	10	25	50
Phosphamidon	Oral	50	25	—	—
	Subcutaneous	50	25	25	—
Shell SD-3562	Oral	100	10	25	25
	Subcutaneous	100	10	25	10
Stauffer R-2371	Oral	>100	50	*	*
	Subcutaneous	100	—	—	—
Stauffer R-2448	Oral	>100	50	*	*
	Subcutaneous	100	—	—	—
Virginia-Carolina 1–13	Oral	>100	10	25	*
	Subcutaneous	100	—	—	—

[a] Not toxic to arthropods at highest dosage not lethal to guinea pigs.
[b] Not toxic to arthropods at highest dosage administered to guinea pigs.

lineatum and *bovis*) begin to appear in their backs in December to January. These animals are usually treated in November when enough grubs have appeared in southern cattle to indicate effective treatments.

After treatment the animals are maintained on pasture and examined monthly for encysting grubs. Cumulative counts of grubs are obtained by marking the location of grubs on an outline map of the back of each animal. A certain number of animals in each group are not treated, and control is based on differences in the number of grubs from treated and untreated animals.

The results of seven tests with 18 compounds are summarized in table 2. The average of 36.3 grubs in untreated cattle is based on 20 animals, 45.3 on 9 animals, 38.8 on 21 animals, 16.7 on 25 animals, 22.1 on 9 animals, 20.7 on 10 animals, and 23.9 on 26 animals. Bayer 29493, Dowco 105, and Stauffer R-2371 gave 95% to 100% control at dosages tolerated by cattle; American Cyanamid 18706, Bayer 25141, and Dition were ineffective; and the rest were inconsistently effective or effective at dosages toxic to cattle.

Table 2.—Effectiveness of 18 compounds in tests against grubs in cattle.

Compound	Method of Administration and Dosage	No. of Cattle Treated	Average Number of Grubs in Untreated Cattle	Control (%)
Am. Cyanamid 18706	Drench 15 mg./kg.	2	36.3	0
Bayer 23129	Drench 10 mg./kg.	1	45.3	43
	5	1	45.3	100
	Intramuscular 10	1	45.3	76
	5	1	45.3	0
	Spray 0.25%	4	36.3	94
	.1	4	36.3	52
Bayer 24498	Intramuscular 5 mg./kg.	4	38.8	46
	Capsule 2	2	16.7	82
Bayer 25141	Capsule 0.5	2	38.8	0
Bayer 25142	Capsule 5	1	22.1	100
	4	4	16.7	52
	3	4	16.7	51
	2.5	1	21.1	95
	2.5	4	38.8	85
	Intramuscular 3	4	20.7	41
Bayer 25198	Capsule 10	1	22.1	95
	5	1	22.1	100
	4	4	38.8	65
	2	2	38.8	65
	Spray 0.5%	4	16.7	82
Bayer 29493	Capsule 25 mg./kg.	1	22.1	100
	20	4	16.7	97
	15	2	38.8	77
	10	4	38.8	60
	Spray 0.5%	4	16.7	100
Butonate	Capsule 100 mg./kg.	2	16.7	88
	75	1	38.8	0
	50	4	38.8	36
	41.4	1	22.1	91
	25	1	22.1	0
	Spray 2%	2	16.7	36
Dition	Capsule 15 mg./kg.	1	38.8	0
	8.6	1	38.8	30
Dowco 105	Drench 100	4	36.3	99
	50	4	36.3	97
	25	4	36.3	32
General Chemicals 4072	Capsule 20	2	38.8	68
	10	2	38.8	0
	Spray 0.5%	2	16.7	79
Monsanto CP-10502	Drench 50 mg./kg.	2	36.3	94
	25	2	36.3	100
	20	2	21.1	100
	20	4	38.8	65
	10	2	21.1	100
	Intramuscular 20	1	21.1	79
	Spray 2%	3	16.7	53
	1	4	23.9	74
Monsanto CP-10516	Drench 50 mg./kg.	2	36.3	96
	25	2	36.3	75
	Spray 2%	4	16.7	0
	1	4	23.9	79
Phosphamidon	Capsule 10 mg./kg.	1	22.1	100
	5	1	22.1	55
	Spray 0.5%	1	16.7	93
	.25	1	16.7	70
Shell SD-3562	Capsule 10 mg./kg.	1	22.1	19
	5	1	22.1	100
	5	1	38.8	0
	Spray 0.5%	3	16.7	62
Stauffer R-2371	Capsule 100 mg./kg.	2	20.7	100
	75	2	20.7	100
	50	4	38.8	93
Stauffer R-2448	Capsule 100	4	16.7	97
	50	4	38.8	78
Virginia-Carolina 1–13	Capsule 20	2	22.1	100
	20	3	38.8	67
	10	2	22.1	93
	Drench 20	3	38.8	41
	15	2	36.3	99
	Intramuscular 15	2	22.1	44
	Spray 1%	2	16.7	0

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INDEX XVII

INDEX TO THE LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY

Number XVII of this series has gone to the printer and will be available early in 1961. It covers the literature for 1958. Indexes I through XVI covering the literature from 1905 through 1957 are also available.

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SCIENTIFIC NOTES

Comparison of Sevin with DDT and Rotenone on Tomatoes under Tropical Conditions[1]

GEORGE W. MISKIMEN, *U.S.D.A., Agric. Res. Serv., Virgin Islands Agricultural Program, Kingshill, St. Croix*

Sevin®(1-naphthyl methylcarbamate)[2] has shown promise where tested since its introduction in 1957 (Allen & Rudinsky 1959, Cowan *et al.* 1959, Hofmaster 1959, Long *et al.* 1959).

Tests by its manufacturers indicate broad control action with excellent residual properties. Due to a carbamate chemical basis and consequent different physiological attack, it has been found useful against insects that have acquired resistance to chlorinated hydrocarbons. An added advantage is low toxicity, orally and topically, to man and other warm blooded animals, as well as to fish and their principal food organisms. When Sevin first appeared on the market, it seemed possible that it might be the answer to certain failings of insecticides presently used in the Virgin Islands. Rotenone, DDT, and chlordane, for example, have not been as effective here as elsewhere; rotenone was least effective. Environmental conditions, storage problems, and increasing insecticide resistance all may be factors reducing effectiveness.

A number of crops such as tomatoes, cabbage, broccoli, cucumbers, and squash, otherwise climatically and economically suited to St. Croix are severely restricted by insect and nematode pests. Tomatoes, especially Louisiana All Season and Louisiana Dixie, are known to do well on local heavy-clay soils when planted during the rainy periods of autumn and late spring. However, insects regularly cause extensive damage, especially to fall plantings (see list below). Pentatomids, Coreids, and Agromyzids regularly cause extensive damage. When nematode damage also occurs, production may be reduced to almost nothing. Since tomatoes are a potentially valuable commercial export crop in the Virgin Islands, tests were made during the 1959 fall season on the Louisiana All Season variety to compare the effectiveness of Sevin with DDT and rotenone. DDT and rotenone were selected because they are regularly used on St. Croix by local farmers. Comparative infestation studies of DDT and rotenone had not been previously made so the degree of resistance acquired by local pests cannot be determined.

Many papers on pesticide testing fail to include sufficient local environmental data, making adequate comparison with other similar tests impossible. Therefore, basic ecological information is included in this paper. The area utilized for the test was on the Federal Experimental Station, on unfertilized Fredensborg clay, a moderately heavy, fertile soil. Rainfall during recent years at the Experimental Station has been about 40 inches annually. The real effectiveness of this rainfall is reduced by year round steady winds averaging 10 to 15 miles per hour and by very few completely cloudy days. Annual evaporation rate is approximately 6 feet. Climatological data during the experiment are given in table 1.

PROCEDURE.—The 15-row test area was oriented east and west. The land to the east and west of the experimental area was planted to yams and grass for several hundred feet. Two border rows to the south and four to the north were used as control areas. They contained 70 and 87 plants, respectively. A plot consisting of 100 plants in three rows was set up for each of the three insecticides tested. Each of these plots was subdivided into nine equal subplots to ascertain position effect, which proved to be insignificant.

Seedlings were grown in propagating beds and were transplanted to the test area on August 14, 1959, when 4 to 8 inches in height. Initial application of the insecticides was made on that date with 19 additional treatments at 1-week intervals, the last application being made on December 23. Sevin, 50% wettable powder, or DDT, 50% wettable powder, each at 1 lb. active ingredient/100 gallons water; or 1% rotenone dust, at 0.5 lb. active ingredient/acre, were used in this test. Observations of test plants were made at about 10-day intervals; general appearance, degree of defoliation, amount of flowering, fruit set, and size of fruits being noted.

List of principal insect pests observed on tomatoes on St. Croix, U.S.V.I., during 1959.

Sphingidae	—*Protoparce sexta* (Johan.)
Sphingidae	—*Erinnys ello* L.
Phalaenidae	—*Prodenia ornithogalli* Guen.
Phalaenidae	—*Prodenia latifascia* Walker
Agromyzidae	—*Agromyza* sp.
Pentatomidae	—*Nezara viridula* (L.)
Coreidae	—*Phthia picta* Drury

Table 1.—Climatological data applicable to the Federal Experiment Station, Kingshill, St. Croix, U. S. Virgin Islands, July–December, 1959.

Month (1959)	Rainfall in Inches	Departure[a]	Average Temperature (degrees F.)[b]		Average Mean Departure
			Max.	Min.	
July	3.40	−3.56	87.9	75.7	−0.1
August	4.72	+0.44	88.2	76.1	None
September	2.78	−2.03	89.5	76.6	+2.0
October	2.39	−2.36	88.8	75.7	+1.5
November	5.37	+0.85	86.8	73.9	+1.2
December	2.26	+1.56	86.3	73.6	+2.5

[a] Derived by interpolation of long term readings prepared by the U. S. Department of Commerce Weather Bureau from Bethlehem Upper New Works (1938 to date) and Bethlehem Old Works (1937 to date), one-half mile north and south, respectively, from test areas.

[b] Reading at Alexander Hamilton Airport, 2 miles southwest and 25 feet ower.

RESULTS.—*September 16.*—Untreated plots were nearly stripped of their leaves by large numbers of Sphingid and Phalaenid larvae. Moderate amounts of damage occurred on the DDT and rotenone plots, little difference in damage being observed between the two. Sevin plots were almost untouched. Agromyzid damage was considerably reduced in the Sevin plots compared with all other plots.

September 26.—Untreated areas were recovering from caterpillar depredation which appeared to have run its course. New foliage was appearing but these plants were far behind all others. DDT and rotenone plots were approximately intermediate between the untreated and the Sevin-treated plots. Sevin-treated plants had dense luxuriant foliage and were flowering heavily by this date.

October 6.—Sevin-treated plants had developed large numbers of fruits, many of which were up to 2 inches in diameter. DDT and rotenone plots had just set fruits, untreated had none. Foliage continued superior in the Sevin plots but the remainder of the test was gaining.

October 16.—General observations similar to those of October 6 with fruits on Sevin-treated plants quite large.

[1] Accepted for publication May 9, 1960.

[2] Kindly furnished by Union Carbide Chemicals Company.

Table 2.—Pounds of marketable ripe Louisiana All Season tomatoes harvested during various test intervals.[a]

Treatment	Test Interval						Increase in Yield (%)
	Oct.	Nov. 1–15	Nov. 16–30	Dec. 1–15	Dec. 16–30	Total	
Sevin	14.0	31.3	128.1	46.0	5.2	226.6	441
DDT	0.7	11.0	61.5	40.5	10.0	123.7	242
Rotenone	0.3	1.1	18.1	15.5	14.6	49.6	99
Untreated	—	—	16.3	22.7	12.5	51.4	Base 100

[a] In all cases, adjustment was made for the slight variation in numbers of plants in the plots.

October 26.—Fruits on Sevin-treated plants were ready to harvest while those on DDT and rotenone plots were small and green, being only 1 to 2 inches in diameter. Untreated plants began a small amount of flowering and fruit set during this period.

November 15.—The Sevin-treated plants were producing heavily at this time; DDT and rotenone far behind; untreated still not producing. Tomatoes are customarily not staked on St. Croix, a practice which contributes to heavy ground rot damage that often reaches 50% fruit loss. This factor biases the production figure somewhat against Sevin. Surveys made November 25, December 15 and 30 revealed no noteworthy developments except that the Sevin-treated area continued to produce considerably more tomatoes than other plots. Table 2 shows the yields of the marketable tomatoes harvested.

Discussion and Conclusions.—Under the comparatively dry and warm conditions of the Virgin Islands, between October and December 1959, Louisiana All Season tomatoes treated with Sevin produced 183.2% more fruit than DDT-treated plants and 457.1% more than those treated with rotenone. Treatment with rotenone did not result in any increase in production over untreated plots. Control by Sevin of the principal pests, Sphingid and Phalaenid larvae, was very effective. Agromyzids, apparently not at all controlled by either DDT or rotenone, were at least partially checked by Sevin.

No chemical phytotoxicity was noted in any of the treated plots. Plants treated with Sevin appeared to have a greater set of fruit than any other test group. Rot developed on the fruit on the unstaked plants and caused considerable damage resulting in some bias against the production figures for plants treated with Sevin.

Plants treated with Sevin, even as early as November 30, had produced more than 2.4 times as much harvested fruit as those treated with DDT. Such rapid production is especially advantageous in areas of sporadic rainfall such as occurs on the Virgin Islands.

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Infrared Determination of Aldrin and Dieldrin in Aldrin-Treated Soil[1]

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It has been shown that aldrin in soil is partially converted to its epoxy derivative, dieldrin (Gannon & Bigger 1958, Lichtenstein & Schulz 1959, 1960, Lichtenstein & Polivka 1959, Bolen *et al.* 1958). This conversion was originally suspected because of the consistently poor analytical agreement among total-chloride, bioassay, and specific colorimetric procedures. And it was first demonstrated by use of specific colorimetric procedures after acceptable chromatographic separation. This note describes an infrared evaluation designed to separate, identify, and determine amounts of aldrin and dieldrin in some aldrin-treated soils.

Procedures.—*Aldrin.*—Concentrate an aliquot of *n*-hexane stripping solution, resulting from an isopropyl alcohol—hexane stripping of the soil, to less than 5 ml., then quantitatively transfer it to a 10×150-mm. column of aluminum oxide Woelm, Basic Grade I, and wash with 150 ml. of *n*-hexane. Evaporate the hexane eluate to near-dryness in a Kuderna-Danish evaporative concentrator and remove the last traces of solvent with the aid of a gentle jet of air. Dissolve the residue in 0.3 ml. of spectral grade carbon disulfide and record the spectrum from 1200 cm.$^{-1}$ to 910 cm.$^{-1}$ (8.35 μ to 11.00 μ) in a microcell of 3-mm. light path, with no compensation in the reference beam other than air. The amount of aldrin present is determinable from the peak at 1250 cm.$^{-1}$ (8.00 μ) by means of the baseline technique, with a sensitivity of 200 μg. per 0.1 absorbance unit and no deviation from Beer's law.

Dieldrin.—Elute the aluminum oxide column from above with 150 ml. of chloroform and evaporate as before in a Kuderna-Danish evaporative concentrator. Oxidize the residue with 1 gm. of chromic anhydride in 20 ml. of glacial acetic acid for 10 minutes at 100° C., then dilute the mixture to 200 ml. with water and extract with 150- and 50-ml. portions of *n*-hexane. After combination, dry the hexane solution with anhydrous sodium sulfate and evaporate carefully as before in a Kuderna-Danish evaporative concentrator. Dissolve the residue in 0.3 ml. of spectral grade carbon disulfide and record the spectrum from 1200 cm.$^{-1}$ to 910 cm.$^{-1}$ (8.35 μ to 11.00 μ) in a microcell of 3-mm. light path, with no compensation in the reference beam other than air. The amount of dieldrin present is determinable from the peak at 1180 cm.$^{-1}$ (8.48 μ) by means of the baseline technique, with a sensitivity of 280 μg. per 0.1 absorbance unit and no deviation from Beer's law.

Discussion.—Garhart *et al.* (1952) recommended using the absorption peak at 1180 cm.$^{-1}$ (8.48 μ) for determining aldrin and the absorption peak at 910 cm.$^{-1}$ (10.98 μ) for determining dieldrin. However, the absorption characteristics of carbon disulfide in the long light-path microcell required for the present purpose obviate the use of the 910 cm.$^{-1}$ peak for dieldrin. The aldrin peak at 1250 cm.$^{-1}$ is sharper and more intense than the peak at 1180 cm.$^{-1}$ in the present environment.

This procedure for aldrin incorporates an overall recovery of 92% and responds reliably to 50 μg. of aldrin. The procedure for dieldrin has an overall recovery of 75% and responds reliably to 80 μg. of dieldrin.

Table 1 presents a comparison of residue values found in aldrin-treated soil by the infrared procedure and by the colorimetric procedures for aldrin (O'Donnell *et al.* 1954) and dieldrin (O'Donnell *et al.* 1955) after chromatographic separation. Figures 1 and 2 show the infrared characteristics of aldrin and dieldrin isolated from aldrin-treated soil compared with the infrared characteristics from control-sample extractives of soil.

[1] Paper No. 1220, University of California Citrus Experiment Station, Riverside, California. Accepted for publication June 13, 1960.

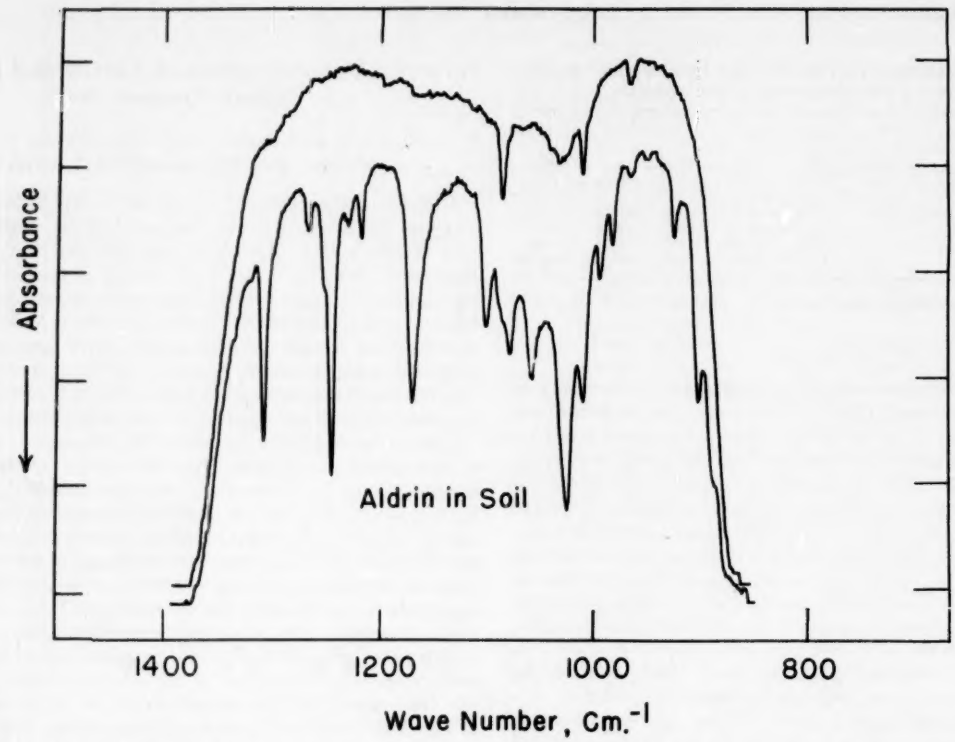

FIG. 1.—Infrared characteristics of aldrin isolated from aldrin-treated soil (lower) and of extractives from control soil (upper). (Perkin-Elmer Model 21 infrared spectrophotometer, resolution 927, response 1 to 1, gain 5.5, speed 2 minutes per micron, and suppression 2.)

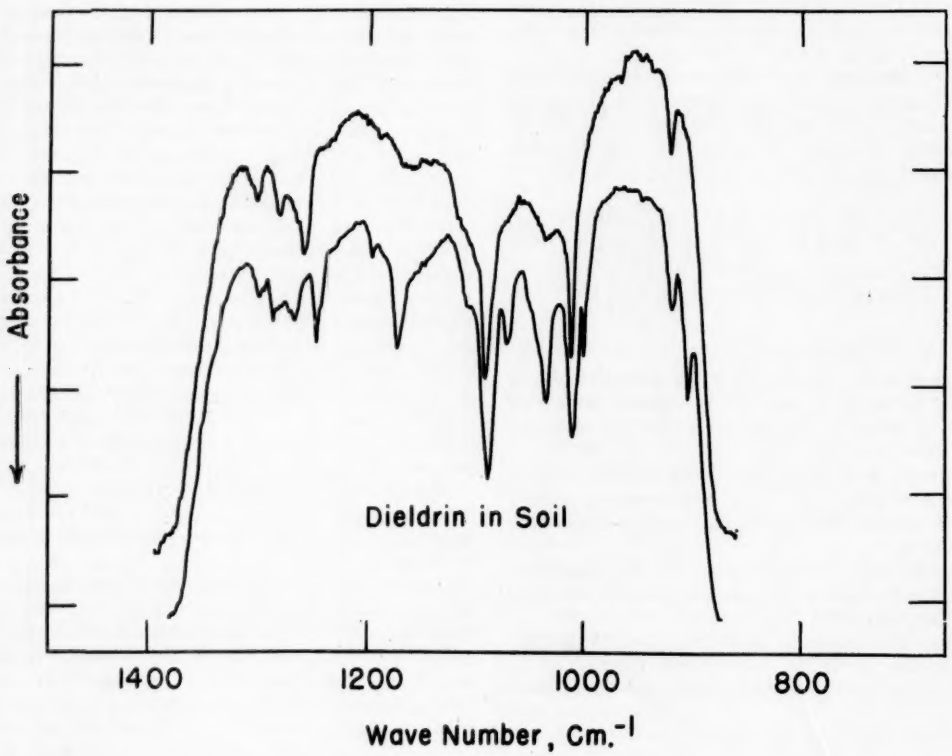

FIG. 2.—Infrared characteristics of dieldrin isolated from aldrin-treated soil (lower) and of extractives from control soil (upper). (Perkin-Elmer Model 21 infrared spectrophotometer, resolution 927, response 1 to 1, gain 5.5, speed 2 minutes per micron, and suppression 2.)

Table 1.—Comparison of residue values, on dry weight basis, found in aldrin-treated[a] soil by infrared and colorimetric procedures.

INSECTICIDE	P.P.M.	
	Infrared[b]	Colorimetric[c]
Aldrin	2.3, 2.0	2.4, 2.7
Dieldrin	0.6, 0.7	0.3, 0.4

[a] Coachella fine sandy soil was placed in stainless steel pans set in soil in a vineyard and the surface of the soil treated with an aldrin granulated (24/48 attapulgite) formulation. The granules were then mixed into the top ¼ to ½ inch of the soil. After 5 days, during which the air temperature maximum ranged from 76° to 99° F. and the soil temperature maximum ranged from 84° to 138° F., the samples were removed for aldrin and dieldrin assay. The aldrin formulation analyzed by infrared procedure was found to contain 4.1% aldrin with no detectable dieldrin.

[b] Corrected for background values from control soil (apparent aldrin, 0.0 p.p.m.; apparent dieldrin, 0.0 p.p.m.) and for recovery (aldrin, 92%; dieldrin, 75%).

[c] Corrected for background values from control soil (apparent aldrin, 0.1 p.p.m.; apparent dieldrin, 0.0 p.p.m.) and for recovery (aldrin, 98%; dieldrin, 99%).

The absorption peaks for aldrin at 910, 932, 990, 1002, 1020, 1035, 1066, 1085, 1110, 1178, 1225, 1237, 1253, 1275, and 1315 cm.$^{-1}$ are in agreement with those found for purified aldrin, and the absorption peaks for dieldrin at 912, 1006, 1042, 1075, 1180, 1200, and 1253 cm.$^{-1}$ are in agreement with those found for purified dieldrin. All extraneous absorption peaks in the spectra from aldrin-treated soil were accounted for by soil extractives; none of the major absorption peaks from the compounds of interest were absent.

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An Effective, Economical Flytrap[1]

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The trap for filth flies here described was devised by the author and John M. Seago in 1952 and tested for several seasons (Dodge & Seago 1954).

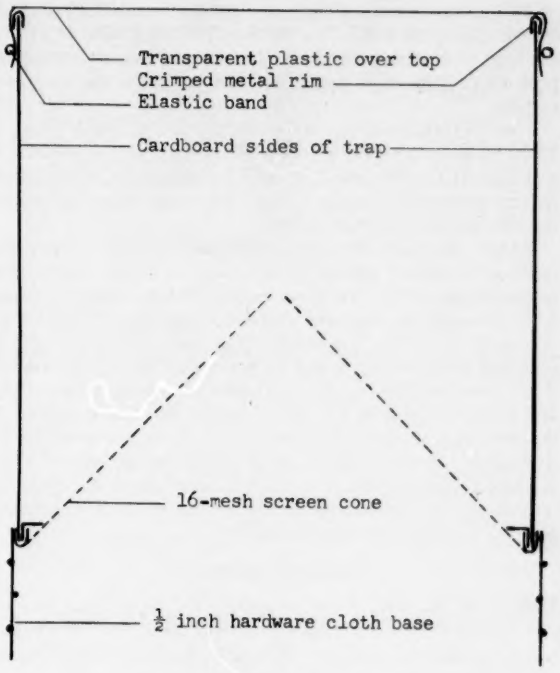

FIG. 1.—Sectional view of trap.

The usual flytrap has a square wooden frame and screened top and sides, or it may be cylindrical, with a galvanized metal frame (Peterson 1953). A cylindrical all-metal trap, about 16 inches in diameter and 16 inches high was used successfully in the U. S. Public Health Service fly studies beginning in 1946. Experiments with a colossal trap 3 feet square at base in 1947 showed that a certain "saturation point" was quickly reached, after which a larger trap would not catch proportionally more flies than an average sized one. A trap that is too small will not catch many of the flies it attracts.

The materials needed to make our trap are a 2½ gallon ice cream carton 9 inches in diameter, with wire-rimmed margins, a 2-inch×30-inch strip of ½-inch hardware cloth, a semicircular piece of 16-mesh wire screen, 18¾ inch-long at base, a soldering outfit, transparent or translucent plastic sheeting and an elastic band. It is advisable to cut a paper pattern and fit it to the trap before cutting the semicircular screen.

To make the trap, a sectional view of which is shown in figure 1, cut the bottom out of the carton leaving an inside edge three-eighth inch wide. Roll the screen into a cone, overlapping the margins one-quarter inch and solder them together. Cut a hole one-half inch in diameter in the top of the cone, place the cone inside the carton and solder the base of the cone into the basal rim of the carton. Then solder the strip of hardware cloth onto the basal rim, to serve as a base for the trap to stand on. Cover the trap with a 12-inch square piece of transparent plastic, secured by a cord or a large rubber band. The cardboard may be painted with one or two coats of aluminum paint to make it more weatherproof, if desired.

This trap is easily and economically made, light in weight and easy to handle. The ¾-inch cardboard margin left in the bottom of the trap prevents flies from lodging in the sharp angle between the cone and the sides.

[1] Accepted for publication July 21, 1960.

[2] This trap was developed and tested while the author was an employee of the U. S. Public Health Service.

To operate the trap, set it over a suitable bait and prop several stones against the base. This will help protect it against wind or molestation by smaller animals. If ants raid the trap change its position to a more favorable one and/or empty the trap each evening.

Flies for taxonomic purposes or population studies may be killed by coating the inside walls of the trap with a residual insecticide. If the flies are to be used for virus tests, remove them by first placing the trap in a larger container with dry ice until the flies are dead or immobilized.

BAITS.—A meat-based bait is preferred by many, but is susceptible to molestation by predators. It is sometimes possible to place traps in fenced enclosures, but often advisable to change baits if molestation is a problem. A correspondent who operated a trap in Nicaragua learned by trial and error that the only practical baits, owing to the prevalence of dogs, pigs and vultures, were jellyfish or pig excrement. Common protein baits are chicken entrails or fish heads, usually procurable free at the nearest meat market. Watermelon rinds are an excellent attractant in very dry weather, either alone or as a base on which to place a meat or feces bait. The moisture from the rinds also retards desiccation of such baits. In wet weather watermelon rinds alone are a very poor attractant.

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Longevity of Refrigerated Nosema Spores—*Nosema apis*, a Parasite of Honey Bees[1]

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Nosema disease of adult honey bees (*Apis mellifera* L.) is thought to be the most widespread of all adult-bee diseases. It has been recognized in all beekeeping regions of the world. According to White (1919), Dönhoff first observed microscopic oval bodies in the stomachs of adult bees in 1857 in Germany. Zander recognized these to be protozoan spores and named the species *Nosema apis* in 1909. It belongs to the order Microsporidia. White first saw spores in the United States in 1910. The small oval spores varied in size, but measured about 0.0002 inch in length and somewhat more than half this dimension in width.

Heap (1914) reported that Nosema spores in honey remained infective for at least 2 years. White (1919) performed a wide variety of experiments to determine the longevity of Nosema spores: when heated in water, when suspended in honey solutions, when infected bee stomachs were dried on glass slides and kept at various temperatures, when crushed bee stomachs were exposed directly or in water to sunlight, and when bodies of dead infected bees were kept at various temperatures. He resuspended the infectious material in sugar sirup and fed it to entire colonies of healthy bees to determine whether the spores were still infectious. The maximum longevities White observed were 3½ months in dead bees and 7 months in crushed bee stomachs dried on glass slides, when both types of samples were kept at icebox temperatures. Zander (1921) claimed that Nosema spores remained viable for 5 years but failed to give the evidence on which he based this statement. Morgenthaler (1931) reported that Nosema spores in honey produced a severe infection after 251 days and a slight infection after 312 days. Borchert & von Dehn (1931) found that Nosema spores in dead bees kept at −4° C. and 90% to 100% relative humidity retained their virulence for at least 81 days when fed to individual bees. Similarly, Borchert & Polzin (1933) showed that Nosema spores in water, or dried on glass, were still infective after 93 days. Winkel (1939) determined that Nosema spores in honey kept at 2° to 6° C. retained their viability for 2 years.

The intestinal tract of a bee infected by *Nosema apis* is frequently swollen and discolored. If the bee is alive, or has just died, the entire intestinal tract can be removed as follows: hold the thorax with thumb and forefinger, then grasp the tip of the abdomen with a pair of forceps and pull. A gentle pressure of the thumb and forefinger will help to force the intestinal tract from the abdomen with the pull of the forceps. In a healthy bee the long cylindrical mid-intestine is usually of a reddish-brown color. Circular constrictions show for nearly the entire length of the intestine, and the tissues are fairly tough and of a healthy appearance. A heavily infected intestine usually is a dull grayish-white, and most of the circular constrictions have disappeared. The tissues have become soft and watery, and are more easily crushed than healthy tissues. However, there is considerable variation in the appearance of the mid-intestine of a healthy as well as an infected bee. After the bee is dead a microscopic examination is essential to diagnose the disease. The alimentary tract is removed and the ventriculus is detached from the small intestine. The presence of epithelial cells packed with spores is the criterion for positive diagnosis.

In 1917 the writer worked with Scott & O'Roke (1920) in an attempt to find an intermediate host for the muscle parasite of sheep, *Sarcocystis tenella* Railliet. Honey bees heavily infected with *Nosema apis* were fed to laboratory sheep. The Nosema spores somewhat resembled the protozoan found in the heart muscle of sheep. These experiments indicated that the animals were not infected by the Nosema parasite of bees.

The author's interest in the Nosema parasite was revived in 1942 while he was working at the Bee Culture Laboratory, Laramie, Wyo., and led to the establishment of a Nosema spore "bank" in June, 1953. To establish the bank, in order to learn more about the persistence of *Nosema apis* spores, the author removed the ventriculi from 38 heavily infected bees. The ventriculi, crowded with spores, were placed into sterilized, distilled water to make up the bank and stored in a refrigerator at about 40° F. The spore count of this mixture was about 1,800,000 spores per milliliter. Each year since that time, bees in small screened cages were given 20 to 25 ml. of the spore mixture added to both their sirup and water supply. After all the inoculated food and water were consumed, the bees were provided with a continuous supply of untreated 60% sugar sirup and fresh water. The caged bees were kept at room temperatures throughout the study since Burnside and Revell demonstrated in 1948 that temperatures below 57° or above 93° F. retarded the development of *Nosema apis*. Control bees, obtained from the same colonies, received only sugar sirup and fresh tap water. The food and water were supplied in inverted small glass vials with perforated, aluminum screw tops.

Table 1 summarizes infections and longevities of the bees in the inoculated and control cages each year. The average and maximum longevities in the inoculated cages were calculated for infected bees only. Twenty-five to forty per cent of the inoculated bees escaped infection, all of which died within 13 to 22 days. The percentage that escaped infection tended to increase with the age of the spores fed.

From this study, it is seen that the *Nosema apis* spores are very hardy protozoans. When kept refrigerated, these spores were found to be viable for nearly 7 consecutive years. The

[1] Accepted for publication July 27, 1960.

[2] In cooperation with the University of Wyoming. Wyoming Agricultural Experiment Station, Journal Paper No. 155.

Table 1.—Persistence of Nosema spores from a bank established in 1953—*Nosema apis*, a parasite of honey bees.

Date Bees Inoculated	No. of Bees in Cage		Per Cent of all Inoculated Bees Infected at Death[a]	Days from Inoculation until Death of First Infected Bee	Days from Death of First Infected Bee until All Remaining Bees Were Infected	Length of Life, in Days			
						Average		Maximum	
	Inoculated	Control				Inoculated	Control	Inoculated	Control
Feb. 8, 1954	124	123	75	15	0	31	58	39	76
1, 1955	262	176	72	13	1	22	46	32	63
Mar. 8 1956	255	205	67	14	7	27	38	40	55
Feb. 11, 1957	262	223	60	16	6	17	32	35	51
5, 1958	209	202	66	19	1	30	51	37	72
17, 1959	171	131	63	12	4	21	34	37	53
Jan. 25, 1960	154	164	60	18	5	28	49	33	72

[a] All control bees were negative for Nosema.

first Nosema-infected bees in each cage died in 12 to 19 days after the initial feeding of spores. In these tests the criterion for infection was the presence of spores in the epithelial cells, not just in the stomach lumen. Within 7 days after the first infected bee was found, the remaining dead bees from the inoculated cages showed 100% infection.

The life of Nosema-infected bees ranged from 17 to 31 days. This was 11 to 27 days less than that of their uninoculated sister bees in the 7 years' tests. The range of maximum life for the infected bees was 32 to 40 days. This was 15 to 39 days shorter than the maximum longevity of the control bees.

These tests showed that the percentage of bees becoming infected each successive year tended to decrease slightly (from 75% in 1954 to 60% in 1960). This may not be significant because of the variation in the number of bees in each cage, and the amount of inoculum added to the food and water of each cage, in different years. However, in the last two seasons it was observed that the spores' size, shape, and ability to refract light were changing. They were becoming dwarfed or stunted. Their shape was more oblong and narrow. Many of the spores under the microscope appeared to be dull and to have lost their original freshness. Some of the original spores remained unchanged as far as could be perceived. These external changes of the spore may or may not have changed their virulence to honey bees.

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Effect of Colchicine on Screw-Worms[1]

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Colchicine has long been known for its mutagenic effects on plants and animals. Mitlin and associates (1957) found that female house flies (*Musca domestica* L.) that were fed colchicine were sterilized, but that the fertility of males was unaffected. Since the testes are much better developed than the ovaries at the time of adult emergence, their experiments suggested that male sterilization might be achieved by feeding colchicine to larvae. Tests were conducted at the Kerrville, Texas, laboratory to determine the effect of colchicine on the screw-worm (*Callitroga hominivorax* (Cqrl.)). This insect was used because it was readily available and the colchicine could be uniformly distributed in the rearing medium.

Methods.—The medium used to rear the larvae consisted of 40% ground beef, 40% water, and 20% blood plus 0.2% of 38% formalin (Melvin & Bushland 1940). The colchicine was dissolved in acetone and added to the medium, which was maintained at 35° C. The concentration of the acetone in the medium never exceeded 0.1%. All tests were carried through the complete life cycle. Records on mortality were kept to determine the effects of concentration of colchicine and of the time of treatment.

Newly emerged flies were sexed and mated with flies from the same test, from other tests, or with untreated flies to determine fertility. The adults were not fed colchicine, but were given honey and water. Between 7 and 14 days after emergence, the females were confined three times in small glass vials with fresh ground beef. The eggs they produced were held on moistened paper at 35° C. for 24 hours to determine viability. Microscopic examinations of 4- and 8-day-old flies were made to ascertain the morphological effect of colchicine on the testes and ovaries.

Results.—Preliminary tests established that colchicine is much more toxic to screw-worm larvae than Mitlin and coworkers found it to be to adult house flies. A concentration of 0.0005% caused 100% mortality of newly hatched larvae in 48 hours. Nearly all the newly hatched larvae fed 0.00025% survived for 48 hours, but the same concentration killed 2-day-old larvae within 48 hours. When it was found that 0.0001 and

[1] Accepted for publication April 11, 1960.

Table 1.—Effect of colchicine on larval mortality and adult emergence.

Colchicine Concentration (%)	Test No.	Instar(s) Treated	Larval Mortality (%)	Adult Emergence per 100 Pupae	
				Males	Females
0.0001	1	1, 2, 3	0	0	2
	2	1, 2	0	34	42
	3	3	0	6	6
.0002	4	1, 2, 3	83	0[a]	0[a]
	5	1, 2	0	44	40
	6	3	95	0[b]	1[b]
Control	7		0	35	42

[a] Only 86 pupae available.
[b] Only 10 pupae available.

Table 2.—Results of mating tests to determine viability of eggs (10 males and 10 females per test).

Test Numbers[a]		Average Number of Egg Masses per Female	Viability (%)
Males	Females		
7	7	1.0	80
3	7	1.1	50
7	3	1.1	55
3	3	1.5	50
2	7	.3	65
5	7	.6	70
7	2	.7	80
7	5	1.2	60
2	2	.5	55
5	5	1.5	60

[a] See table 1.

0.0002% colchicine could be used without appreciable mortality of newly hatched larvae within 48 hours, tests with these two concentrations were conducted. The results are presented in table 1. All tests were conducted in duplicate.

Colchicine at 0.0001% produced no significant larval mortality when all three instars were fed on treated medium. When only the first and second instars were treated, there was no effect on adult emergence. Treatment of third-instar larvae resulted in no larval mortality but a high pupal mortality.

Colchicine at 0.0002% caused high larval mortality when all three instars were treated, but when only the first and second instars were fed, there was no effect on either larval mortality or adult emergence. High mortality resulted when third-instar larvae were fed medium treated at this concentration.

The pattern of colchicine toxicity is apparently the reverse of the usual pattern of a poison in which the mature larvae are more resistant. During the third instar the sex organs of the screw-worm develop at a faster rate than during the first and second instars, and the toxicity of colchicine is apparently related to this faster maturation.

The results of the mating experiments are presented in table 2. The viability reduction in eggs from most matings was only slight. Only with females from test No. 4 was there no reduction in viability. There was no consistent difference between the effects on fertility of males and females, or between flies from larvae treated as first and second instars and those from larvae treated only as third instars.

Microscopic examination of 4- and 8-day-old flies from larvae treated during the first and second instars with 0.0002% colchicine showed definite morphological differences. The ovaries were less than one-third normal size; this was the same effect noted with adult house flies (Mitlin *et al.* 1957). The effect on the testes was less noticeable. In some flies the normal bright orange color was much paler or nearly white. In others one or both testes were partly degenerated. The reduced size of the ovaries and degeneration of the testes are apparently related to the reduction in viability of eggs.

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The Effect of Heptachlor and Toxaphene on Stand of Ladino Clover[1]

H. A. Fribourg and W. W. Stanley[2]

In May 1956, an experiment consisting of two treatments in a randomized complete block design with four replications was initiated on an established tall fescue-ladino clover sod growing on Cumberland loam soil near Knoxville, Tennessee, with the objective of ascertaining possible effects of soil insecticides on forage plant stands and longevity. Each plot measured 10 by 100 feet. One plot in each replication received 120 pounds per acre of 2.5% heptachlor granules (3 pounds per acre of actual heptachlor); the other plot was not treated. The plots were harvested with a mowing machine, three times in 1956 and seven times in 1957. There were no significant differences in either forage yield or botanical composition between heptachlor-

Table 1.—Effect of insecticide applications on botanical composition of ladino clover-tall fescue sods, Cumberland loam, 1956–1959.

Category and Year	Per Cent Ladino Clover in Stand	
	April	October
1956		
Heptachlor	—	10
Untreated	—	10
1957		
Heptachlor	10	30
Untreated	10	30
1958		
All heptachlor treatments	50	75
All untreated	50	40
1959		
Heptachlor	29	20
Heptachlor+demeton	28	15
Heptachlor+toxaphene	22	32
Heptachlor+Di-Syston	34	10
No heptachlor	12	2
No heptachlor, +demeton	11	8
No heptachlor, +toxaphene	15	34
No heptachlor, +Di-Syston	10	9
L.S.D. for 1959 data at 5% level	12.0	12.6

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FIG. 1.—Effect of heptachlor applied in May 1958, on botanical composition of ladino clover-tall fescue sods on June 19, 1959. Left: heptachlor-treated; right: untreated. Note the larger number of clover blossoms in the plot on left.

treated and untreated plots in either year. Botanical composition data, obtained by visual estimates of per cent ground cover, and checked by actual hand separations, are presented in table 1.

In April 1958, each plot was split into four equal areas measuring 10 by 25 feet. Plots that previously had been treated with heptachlor received another application of 3 pounds of actual heptachlor per acre, using the 10% granular formulation. Three split-plots in each of the heptachlor-treated and untreated areas were treated with either demeton, toxaphene or Di-Syston® (*O,O*-diethyl *S*-2(ethylthio)ethyl phosphorodithioate). Demeton was applied at the rate of 1.5 pint per acre (25% formulation) in April 1958, and five times in 1959, approximately at 1-month intervals and beginning in April. Fifty pounds per acre of 2.5% granules of Di-Syston were applied to the sod in April 1958, and again in April and June 1959. Two pounds of toxaphene were applied by spraying on three occasions in 1958, at 10-day intervals in April; and 14 times in 1959, at 10-day intervals, starting on April 6.

The plots were clipped five times in 1958 and eight times in 1959. There were no significant differences in forage production among any of the treatments either year. However, the botanical composition of the sods was greatly affected by insecticidal treatment (table 1). In the spring of 1958, ladino clover was uniformly distributed among plots and accounted for half the stand. By October, those plots treated with heptachlor had 75% clover stand, whereas the clover stand in the areas not treated with heptachlor had decreased slightly. Demeton, toxaphene, and Di-Syston had no effect on clover stand in 1958.

In 1959, the plots that had been treated with heptachlor the previous year continued to have a better clover stand than those that had not been treated (fig. 1). The heavy applications of toxaphene resulted in a marked improvement in clover stand, whether heptachlor had or had not been applied.

The tall fescue-ladino clover sods were productive throughout the duration of the experiment. Total seasonal dry matter yields ranged between 1.2 and 2.2 tons per acre, depending on rainfall. Nitrogen fertilizer was applied once only (100 pounds per acre of ammonium nitrate) in May 1956, two years before the shifts in botanical composition began. Phosphate and potash were applied as 0–20–20 on three occasions: 250 pounds per acre in September 1957, 200 pounds per acre in April 1958, and 300 pounds per acre in February 1959.

The results indicate that clover stands were influenced in both 1958 and 1959 by the application of heptachlor in 1958, and by toxaphene in 1959, but not by heptachlor in 1956 and 1957. This lack of effects the first two years may have been owing to the small clover percentage of the sod. In 1958, clover growth had been encouraged through repeated clipping of the fescue sod the previous year, and by favorable soil moisture conditions. Prior to that time, the amount of clover in the stand was not large enough for differences to occur or to be of an observable magnitude. The stand of fescue was not affected by treatments and provided essentially the same amount of clover throughout the experiment. Thus, changes in total cover reflected increases or decreases in ground coverage by clover rather than by fescue. Since no insect counts were made, the effect of these broad-spectrum insecticides on insect populations is not known. Whether these chemicals resulted in better clover stands through reduction of insect populations, or whether they stimulated clover growth or depressed fescue growth, remains to be investigated.

A Comparison of the Effectiveness of Aldrin Seed Treatment for Rice Water Weevil Control on Water- and Drill-Seeded Rice[1]

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The use of insecticides as seed treatment for rice water weevil, *Lissorhoptrus oryzophilus* Ksl., control is becoming a common practice for rice farmers in Texas who are concerned with this insect. Previous research conducted with seed treatment as reported by Bowling (1957, 1959) was with drill-seeded rice. An estimated 20% of the total rice acreage in Texas is planted by dropping presprouted seed into flooded fields by airplane. The seed are sprouted by submerging sacks of seed in canals and

[1] Accepted for publication June 27, 1960.

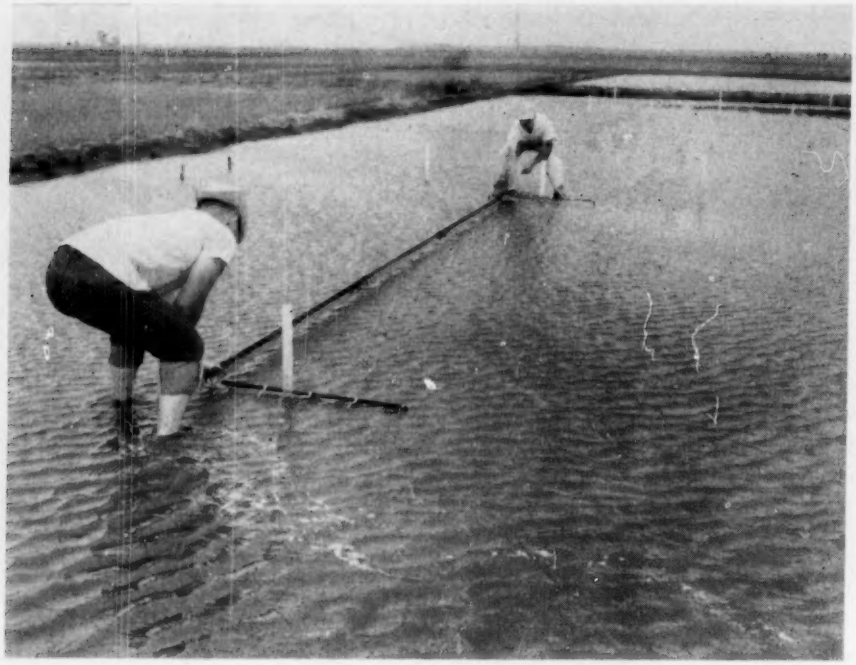

FIG. 1.—Seeding rice in rows using a simulated water-seeding method.

ponds. After 24 hours the seed are removed from the water and germinated an additional 24 hours before seeding. A test was conducted in 1959 at the Rice-Pasture Experiment Station, Beaumont, Texas, to determine if aldrin is retained in sufficient quantities to effect adequate control of rice water weevil when treated seed are planted by the water-seeding method.

EXPERIMENTAL PROCEDURE.—Century Patna 231 seed rice was treated with 50% aldrin wettable powder as a slurry at 4 and 8 ounces technical per 100 pounds of seed. Experimental plots 4′×20′ were established by drill-seeding and by a simulated water-seeding method using the two rates of insecticide applications and an untreated check. The six treatments were randomized within each of four replications and the seeding rate in all was 80 pounds per acre. The seeding rate for the water-seeded plots was kept constant with the drill-seeded plots by catching and weighing the seed that flowered through the drill spouts as the planter moved 20 feet. The drill-seeded plots were planted on June 17, and the entire test block was flooded immediately with 4 inches of water. The seed for the water-seeded plots were sprouted by wrapping the amount for each individual row in a paper towel. The towels containing seed were submerged in water for 24 hours in separate containers. The seed for the water-seeded plots was dropped into the water on July 18, one row at a time, using the device shown in figure 1. The device consisted of one 22′ piece of 1½″ angle iron and two pieces of ½″ concrete reinforcement rods. The pieces of reinforcement rod were marked at 8″ intervals to provide the same row spacing as the drill-seeded plots. One was placed at each end of the plot to be planted. The sprouted seed for one row was distributed evenly by hand in the angle of the 22′ angle iron. The angle iron holding the seed was placed in position over the plot and slowly turned over, dumping the seed into the water in a straight line. Twenty-four hours after the water-seeded plots were planted the test block was drained. This simulated method of water-seeding provided rice growing in plots and rows comparable to drill-seeded rice. Three weeks after planting the plots were fertilized with 33% ammonium sulfate at the rate of 100 pounds of nitrogen per acre. The block was flooded immediately after making the fertilizer application and remained flooded until drained for harvest. Three weeks after flooding, rice water weevil larval counts were made using the method described by Bowling (1957). Yields of rough rice were determined by harvesting the entire plots.

RESULTS AND DISCUSSION.—Larval counts presented in table 1 indicate that both the 4- and 8-ounce application rates of aldrin were equally effective in reducing larval populations whether the rice was water- or drill-seeded.

Yield data given in table 2 indicate that highly significant increases over the check were obtained with both the 4- and 8-ounce rates of aldrin. The yield obtained at the 4-ounce rate was greater than the 8-ounce rate, but the difference was not significant at the 5% level. In this test the yield from water-seeded rice was significantly higher than drill-seeded.

During the process of planting the water-seeded plots it was noted that the treated seed sank to the soil surface more readily than the untreated. As a result of this fact the rows of plants in plots water-seeded with treated seed were much more defined than the rows in the untreated checks. This reduction in drift of seed was probably caused by the wetting agent in the wettable

Table 1.—Number of larvae of the rice water weevil per foot of drill row.

METHOD OF SEEDING	OUNCES TECHNICAL ALDRIN PER 100 POUNDS SEED			AVERAGE
	0	4	8	
Water-seeded	34.0	5.2	9.4	16.2
Drill-seeded	40.2	4.0	2.7	15.6
Average	37.1	4.6	6.0	
	Rates	*Methods*	*Rates* × *Methods*
L.S.D. at 5% level	10.8	N.S.	N.S.
at 1% level	15.0	N.S.	N.S.

Table 2.—Yields of rough rice in pounds per acre.

Method of Seeding	Aldrin, Ounces Technical per 100 Pounds Seed			Average
	0	4	8	
Water-seeded	2,964	3,725	3,394	3,361
Drill-seeded	2,788	3,389	3,277	3,151
Average	2,876	3,557	3,335	

	Rates	*Methods*	*Rates* × *Methods*
L.S.D. at 5% level	244	201	N.S.
at 1% level	339	278	N.S.

powder and could be of benefit when rice is water-seeded on a windy day.

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Laboratory Study of Toxicity of Imported Fire Ants to Bluegill Fish[1]

H. B. Green[2] and Ross E. Hutchins[3]

During late March of 1960 a considerable number of bluegill bream, *Lepomis* sp., were reported dying in farm ponds in South Mississippi and Alabama. Upon examination it was found that several grams of imported fire ants, *Solenopsis saevissima* v. *richteri* Forel, were in the stomachs of these fish (fig. 1) and it was assumed that death of the fish was caused by the ants. This condition followed heavy rains that are known to float masses of ants from their mounds. Residents in the area state that this occurs each spring.

During April and May large numbers of fire ants were thrown into ponds in the vicinity of State College. Although it was observed that fish appeared to feed readily upon the ants, often taking dozens at a time as the ants clung together on the surface of the water, there was no evidence at that time of any abnormal behavior of the fish, and none was found dead.

It was then decided that perhaps more could be learned under laboratory conditions where the reactions of the fish could be more closely studied. Ten- and fifteen-gallon aquaria with compressed air supplies were obtained, and 50 fish ranging from 0.1 to 0.3 pound were introduced. Freshly collected grasshoppers were fed to the fish daily. Most of these fish became rather docile in 2 or 3 days and fed freely when food was dropped upon the surface of the water.

Fire ants were collected in jars and cast into the water of one aquarium while grasshoppers were fed to the adjacent aquarium. It was found that if the fish in one aquarium could see the fish in the adjacent aquarium feeding, they were stimulated to feed on less desirable food or on almost any small object cast into the water. In this manner fish were seen to take ants singly or in floating masses, often a dozen or more. It was not possible under these conditions to tell if the fish had actually eaten the ants, since in most cases the fish were seen to disgorge ants after they returned to their position at the bottoms of the aquaria. In many instances the fish acted as though they had been stung by the ants and seemed to have trouble shaking them from their mouths. After the first encounter with the ants, little or no feeding took place, and no fish died during these tests. Since stinging might have been the cause for lack of feeding, new fish were tested using ants that had been freshly killed by heating the holding jar. In these tests the fish were again seen to disgorge ants, but no sting reaction was noticed. Again no fish died during the tests, and feeding on the ants was negligible. It was therefore assumed that feeding on sufficient fire ants to cause mortality to bluegills must occur under some conditions other than those used in the tests. Fish are known to strike more readily during spawning which is usually heaviest during the spring. At this time, also, other foods are less plentiful. This may account for the large numbers of fire ants found in the stomachs of the fish found dead in early spring.

It was then decided to attempt to force-feed the ants to the test fish. Five grams of ants were macerated in 20 ml. of water using an electric blender. One or 2 ml. of this material was then pumped into the stomachs of five fish, using a long pipette with a bulb to reach into the stomach. Upon release, most of these fish were seen to disgorge much of the material into the water. Within 5 minutes, however, the treated fish showed signs of poisoning. The first noticeable symptom was an inability to maintain equilibrium. There was a tendency to settle to the bottom and then to gradually list to about 45 degrees from vertical. This reaction was usually followed by about 10 minutes of intermittent spasms with extreme activity, with the fish swimming about the aquarium and often causing considerable surface disturbance. All of the treated fish were dead in about 45 minutes. As a check for possible trauma involved in the technique, five other fish were given the same quantities of a similar preparation but using macerated grasshoppers instead of fire ants. These fish did well for several hours and then developed symptoms similar to those of the first group and died after 6 to 8 hours. Since they were placed in the same water that the fire ant treated group had been in, it was assumed that this might have caused their deaths. More fish were then force-fed with macerated grasshoppers and placed in clean water. No mortality occurred, so it was assumed that the contaminated water in the first test had killed the fish fed with grasshoppers.

To test the effect of the macerated ants in water further, several guppies were placed in small beakers. It was found that several drops of the fire ant preparation previously described caused death very quickly. Since this preparation contained a great deal of chitin and other solids, it was filtered to allow better observation of the guppies. It was found, however, that when the macerated fire ants were filtered little or no poisoning symptoms were caused. Some of the filtered preparation was then pumped into the stomachs of bluegills with similar reaction. One-half ml. of the filtered preparation was then injected into the body cavity of bluegills with no ill effects noticed after 24 hours. Bluegills were then placed in 1-gallon jars and filtered and unfiltered fire ant material was added. They reacted in the same way as the guppies, and it was therefore concluded that the toxic substances were not water soluble.

One lot of the macerated fire ants was strained through a 30-mesh screen to remove the larger particles, and 0.5 ml. was then injected into the body cavity of the fish. This quickly caused death in the usual manner. A similarly strained preparation of macerated grasshoppers was injected with no apparent ill effects.

Acetone and alcohol extractions were then made using 5 grams of ants with each solvent with maceration in the blender. These extractions were then filtered and the solvents allowed to evaporate with slight warming and air movement. Three ml. of water were then added to each extraction, and 0.5 ml. of each

[1] Partial cost of publication of this paper was met by the Mississippi Agricultural Experiment Station. Accepted for publication September 16, 1960.

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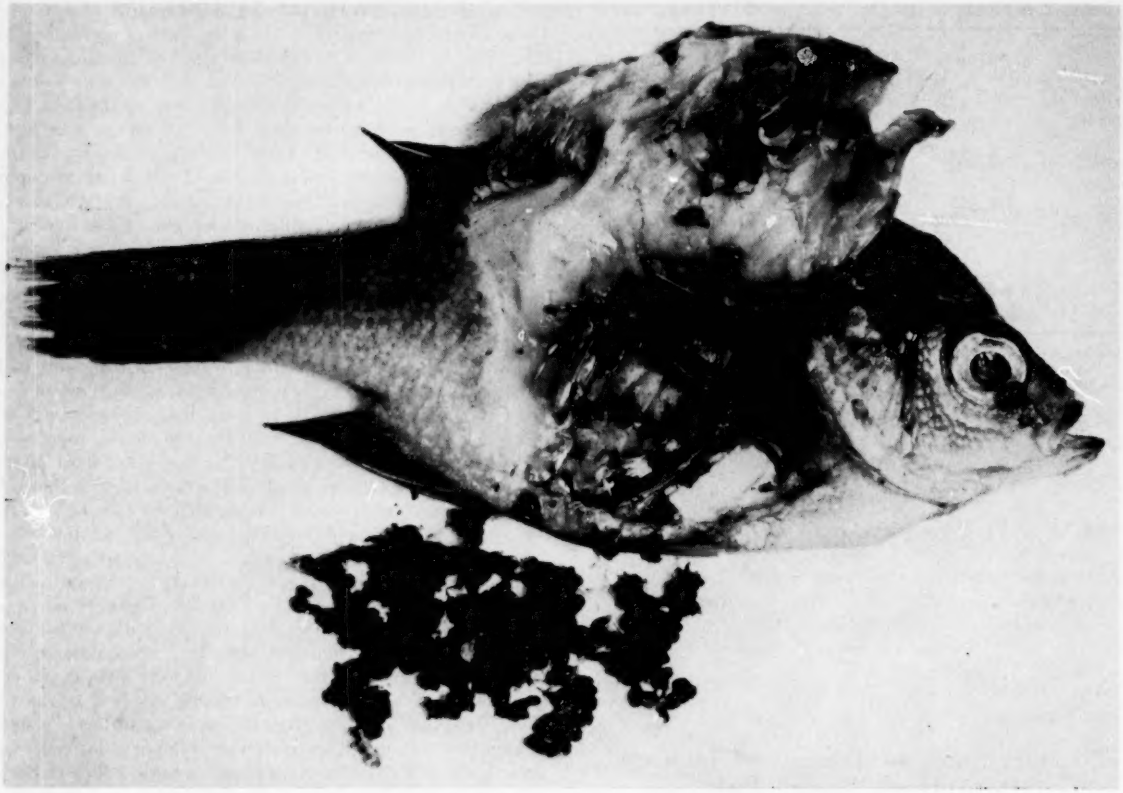

FIG. 1.—A fish, probably killed by eating fire ants. When dissected the stomach was distended by a large ball of fire ants.

was then injected into the body cavities of bluegills. Death resulted quickly.

It is therefore concluded that the venom is not water soluble but is soluble in acetone or alcohol. This confirms results of research done at Louisiana State University by Blum *et al.* (1958). From the above we conclude that when bluegills feed upon imported fire ants in sufficient quantity it is probable that they will be killed. At present it is not known what conditions induce feeding upon the ants in the wild state.

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Development of Chlordane and Malathion Resistance in the German Cockroach[1]

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The broad geographical distribution of resistance in the German cockroach (*Blattella germanica* (L.)) to the chlorinated hydrocarbons (Brown 1958), has made it necessary to use organophosphorus insecticides to combat infestations of this insect. The currently recommended organophosphorus insecticides do not exhibit the residual persistence of the chlorinated hydrocarbons, but they are effective with proper formulation and application. Resistance in field strains of German cockroaches has not yet been demonstrated, although there have been reports of unsatisfactory control, and resistance to malathion has been developed in laboratory strains (Burden *et al.* 1959, Grayson 1960).

In 1956 laboratory experiments were undertaken to study the possibility of preventing or retarding the development of resistance in the German cockroach when exposed to chlordane and/or malathion in each generation. Four experimental colonies were established from a normal laboratory colony by exposure of nymphs in a wind tunnel to contact sprays as described by Burden *et al.* (1959). Colony C was exposed to chlordane, colony M to malathion, and colony CM-C to a combination of chlordane and malathion in each generation, whereas colony CM-A was exposed in every other generation to chlordane and in the alternate generations to malathion. The concentrations of insecticides in the sprays were adjusted to cause approximately 50% to 75% mortality to each generation; however, this selection rate was not always attainable owing to the everchanging susceptibility of the colonies.

Nymphs of each colony were exposed to the insecticides in deodorized kerosene as noted in the concentrations on the following page.

The development of resistance in the colonies was determined in each generation by testing adult males which had not been exposed as nymphs to the usual selection with chlordane or malathion. The male cockroaches were subjected to continuous exposures on residues of 10 mg. of chlordane or malathion per square foot in pint glass jars, according to the method described by Keller *et al.* (1956). The number of cockroaches dead or

[1] Accepted for publication August 19, 1960.
[2] J. L. Eastin, B. J. Smittle, and T. A. Fulton assisted in these studies.

Table 1.—Development of resistance to chlordane and malathion in four experimental colonies of the German cockroach.[a]

Generation	Ratios of Indicated Colonies to Normal Colony								Normal Colony	
	C Colony		M Colony		CM-A Colony		CM-C Colony			
	LT-50	LT-90	LT-50	LT-90	LT-50	LT-90	LT-50	LT-90	LT-50 (hours)	LT-90 (hours)
					Chlordane					
1	1.5	2.2	—	—	2.5	4.4	1.4	1.8	4.3	5.2
2	5.1	10.6	—	—	2.7	6.0	—	—	4.3	5.1
3	5.8	13.4	—	—	3.4	6.6	1.7	2.8	3.8	4.7
4	4.1	13.3	0.9	0.8	1.7	3.0	.8	.7	4.2	5.5
5	9.3	22.4	.8	.8	3.9	8.7	1.3	2.1	4.9	5.4
6	9.7	26.4	—	—	6.9	20.4	3.6	21.0	5.1	6.2
7	19.1	27.3	—	—	5.2	11.9	4.4	6.3	4.9	6.3
8	13.3	27.1	—	—	4.1	11.8	2.9	9.5	4.3	5.1
9	21.2	24.1	.9	1.0	5.4	16.2	11.7	37.4	4.2	5.1
10	20.8	25.3	.8	.9	10.1	19.1	14.5	32.0	3.6	4.2
11	44.4	56.6	1.1	1.1	28.4	31.5	27.8	44.5	3.6	4.7
12	20.2	24.5	1.4	2.2	7.8	13.9	19.5	46.0	4.3	5.0
13	—	—	1.8	2.8	16.7	37.4	25.6	68.2	3.4	4.2
14	—	—	1.2	3.3	8.3	18.2	—	—	5.5	6.1
					Malathion					
1	—	—	1.1	2.7	1.5	3.7	1.6	2.5	1.4	1.9
2	—	—	2.5	4.9	1.6	2.0	—	—	1.6	2.9
3	—	—	.8	.9	—	—	.9	.6	1.8	3.6
4	—	—	3.3	1.8	2.3	.8	2.5	.8	1.3	6.6
5	—	—	5.8	4.5	1.9	1.4	4.0	2.5	1.4	5.2
6	—	—	6.3	11.0	3.6	5.0	2.8	4.1	1.2	2.1
8	—	—	4.9	4.3	5.8	4.5	2.6	5.5	3.2	8.2
9	1.0	0.5	6.3	4.6	5.0	3.2	2.1	.9	1.9	8.0
10	1.3	.8	8.0	5.3	4.2	3.5	2.8	1.7	1.1	2.9
11	.8	.8	7.4	8.8	2.3	3.0	2.2	2.1	1.3	2.4
12	.9	1.7	6.5	4.8	1.2	1.0	3.0	1.7	1.2	2.7
13	—	—	4.8	5.3	1.7	1.7	3.0	2.4	1.3	2.2
14	—	—	8.9	6.5	3.1	1.9	2.0	1.3	1.9	4.4

[a] Two to four replications with 5 to 10 adult males each.

knocked down after different periods of exposure was recorded, and from these data the time required to give 50% or 90% kill or knockdown (LT-50 or LT-90) was computed.

Table 1 shows the LT-50 and LT-90 of the normal colony in each generation, and the ratio of the LT-50 and LT-90 of each resistant colony to those of the normal colony. The LT-50's and LT-90's fluctuated from generation to generation, but the ratios indicated that resistance to chlordane developed as rapidly in the CM-A colony as in the CM-C colony, and almost as rapidly as in the C colony, with resistance of 20-, 21-, and 26-fold being attained by these colonies, respectively, in the F_6 generations.

The CM-A and CM-C colonies developed resistance to malathion at nearly equal rates, showing 2- to 5-fold resistance from the F_6 through the F_{14} generations. The M colony developed resistance faster and to a greater degree—3- to 11-fold resistance in the F_4 through the F_{14} generations. The F_{12} through the F_{14} generations of the M colony exhibited a 2- to 3-fold cross-resistance to chlordane but the C colony did not show cross-resistance to malathion.

These results indicate that the alternate or combined use of chlordane and malathion may retard slightly, but certainly will not prevent, the development of resistance to each of these insecticides.

Generation	*Chlordane* %	*Malathion* %
	Colony C	
P–F_3	0.5	—
F_4	1.0	—
F_5–F_6	2.0	—
F_7	4.0	—
F_8–F_{10}	8.0	—
F_{11}	16.0	—
F_{12}–F_{14}	32.0	—
	Colony M	
P–F_3	—	1.0
F_4	—	2.0
F_5–F_{13}	—	4.0
F_{14}	—	6.0
	Colony CM-A	
P–F_3	0.5 or	1.0
F_4–F_5	1.0 or	2.0
F_6–F_{13}	2.0 or	4.0
F_{14}	3.0	—
	Colony CM-C	
P–F_3	0.5 plus	1.0
F_4–F_{11}	1.0 plus	2.0
F_{12}–F_{13}	2.0 plus	4.0
F_{14}	3.0 plus	6.0

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BOOK REVIEWS

A Review of the Biological Control of Insects and Weeds in Australia and Australian New Guinea. by Frank Wilson. Commonwealth Institute of Biological Control, Ottawa, Canada, Technical Communication No. 1, 102 pp. 1960. Price 25 shillings ($3.75).

This publication is one of a series, sponsored by the Commonwealth Agricultural Bureau, that is intended to survey the work and results in biological control in the different countries of the Commonwealth. It is a comprehensive review of work covering a period of more than 60 years, and deals with more than 50 insect pests. Data are given on the dates of importation and countries of origin of the various imported parasites and predators, the extent of the colonization program and an evaluation, when possible, of the outcome of the program against each pest. The most successful of these have been on *Eriosoma lanigerum* (Hausm.), *Eulecanium persicae* (F.), *Nezara viridula smaragdula* (F.), *Pieris rapae* (L.), *Plutella maculipennis* (Curt.), *Pseudococcus* spp. and *Trialeurodes vaporariorum* (Westw.) in Australia, *Asterolecanium variolosum* (Ratz.), *Eriosoma lanigerum* and *Typhlocyba froggatti* Baker in Tasmania and *Promecotheca papuana* (Cziki) in New Britain and New Ireland.

The account of the early work on scale insects and aphids points up a deficiency that existed not only in Australia but in this country and others as well, that being a failure to obtain identifications of many of the natural enemies that were imported or to maintain an adequate record of field releases.

The section on biological control of weeds records the importation and release of insect enemies against seven species. The largest project, which was spectacularly successful, was that on prickly pear (*Opuntia* spp.), where millions of acres were cleared of the pest through use of the moth, *Cactoblastis cactorum* (Berg.). Effective control of St. John's wort (*Hypericum perforatum* L.), was accomplished in many areas through leaf-feeding beetles (*Chrysomela* spp.).

All biological control workers will appreciate the author's painstaking review of the literature and of information from various other sources. The bibliography comprises 558 titles.

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Ticks—A Monograph of the Ixodoidea. Part V (On the Genera *Dermacentor, Anocentor, Cosmyomma, Boophilus and Margaropus*). by D. R. Arthur. Cambridge University Press, England, xvii+251 pp., 510 figs., 3 pls. 1960. Price $11.50.

The appearance of this excellent contribution to our knowledge of the ticks of the world has delighted arachnologists. Ticks are so important as parasites and vectors of disease of the higher forms of animal life including man that one wonders why this Monograph started 50 years ago by the illustrious Dr. Nuttall and his associates (Warburton, Robinson and Cooper) was not completed long ago.

Dr. P. Tate, Director of Moltino Institute of the University of Cambridge presents a foreword that appropriately recognizes the difficult task admirably carried out by Doctor Arthur in producing Part V of this monumental Monograph.

The present volume deals largely with the genus *Dermacentor* but also includes the genera *Anocentor, Cosmiomma, Margaropus* and the important *Boophilus.*

Brief descriptions are given of the known stages, and available information on the biology and disease relations is summarized. One new species, *Dermacentor asper,* is described from two male specimens from China.

In the forepart of the book appears a complete list of the 510 figures in the text and a brief introduction which includes an explanation of terms used. Following the citation of diagnostic characters of the genus *Dermacentor,* 30 pages and 52 figures are devoted to an excellent discussion of its morphology. The *Dermacentors* are considered in regional groups—American species, of which 11 are recognized, Eurasiatic species 16 and African species two and one variety. Keys to males and females are presented under each regional group rather than under the genus as a whole, which arrangement has some disadvantages. Unfortunately the immature stages are too poorly known for the author to give keys except to American species.

The genus *Anocentor* contains only one species, *nitens.* This form was considered a *Dermacentor* until 1937 when Schulze set up the genus *Anocentor.* The genus *Cosmiomma* was also erected by Schulze in 1919 for the peculiar species *hippopotamensis. Boophilus* contains three species including *annulatus,* the infamous transmitter of so-called Texas Fever. The recognition of only three species in this genus appears logical and is in accord with the ideas of several present-day workers. The genus *Margaropus* has but two species both from Africa.

In a work of this condensed character, the omission of complete distribution and other records is expected. A few such omissions are noted, for instance on pages 206 and 213 reference is made to the occurrence of *Boophilus microplus* in Southern Florida but its presence in south Texas and in Mexico is not mentioned. In this connection, reference is omitted to a short article by me (Cir. 136 Bur. of Ent. U.S.D.A., 1911) reporting on an intensive survey of the distribution of the spotted fever tick, *Dermacentor andersoni,* in the United States.

Under the heading "Species condemned, doubtful, insufficiently described or material insufficient," some 20 species are listed with descriptive notes and remarks. It's a pity that a number of these forms or names could not have been disposed of with greater finality.

The simple but accurate line drawings will be helpful to taxonomists and others concerned with tick problems, and the bibliography of 250 references will be very useful.

Fred C. Bishopp

Chemical and Natural Control of Pests. by E. R. de Ong. Reinhold Publishing Corporation, 430 Park Avenue, New York 22, N. Y. 1960, 244 pp., 12 illus. Cloth, $7.50.

Pest control, the author points out, is primarily natural control exerted by numerous agencies and conditions including beneficial insects and diseases, unfavorable weather and varying food supplies. Usually, however, where the protection of economic plants is involved, these factors leave a sufficiently large remnant of the pest species to require artificial control measures including the employment of biological agents or chemicals.

A concise discussion of biological control is given, involving the use of insect parasites and predators and also certain fungus, bacterial, virus and protozoal diseases. In this connection the effects of numerous widely used pesticides upon beneficial insects and vertebrates are considered and the discussions documented by appropriate reference to published results of experimental work. Included in this section also is a discussion of the development of varieties of certain grain and forage plants, cotton, sugar beets, tobacco, beans, cucurbits, potatoes and other crops, that may be resistant, at least for a time, to specific diseases or insects.

The larger part of the book, however, deals with chemical control since it is most frequently a necessary supplement to natural and biological control. This section begins with a chapter containing a general discussion of inorganic and organic compounds as insecticides, bactericides, fungicides, herbicides or rodenticides, and the use of toxic materials as baits and repellents. Following are chapters treating certain pests (insects,

diseases, weeds) of specific field crops, of orchards and vineyards, of ornamentals and lawns, of vegetable crops, of livestock, of the household and of stored products. In each case control recommendations are given, including the use of the newer chemicals that have shown promise. There is also a helpful appendix which lists the more widely used or more promising pesticides by common or brand name followed, in each instance, by the chemical name and function of the pesticide.

This book is a well-balanced and concise work giving briefly, but clearly and authoritatively, an up-to-date review of the whole field of natural and chemical pest control. It is certain to prove useful.

C. F. W. MUESEBECK,
U. S. National Museum

BIOLOGICAL AND CHEMICAL CONTROL OF PLANT AND ANIMAL PESTS. Edited by L. P. REITZ. xii+285 pp. illus. Publication No. 61 of the American Association for the Advancement of Science, Washington, D. C. 1960. Price $5.75; $5.00 prepaid to AAAS members.

The material in this book was presented in a symposium on Agriculture at the Indianapolis meeting of the American Association for the Advancement of Science in December, 1957. It brings together for the first time many interesting and current subjects on pest control each briefly summarized by an expert in the field. The broad scope of this book, which includes weed control as well as the control of insects and diseases of plants and animals, is a desirable feature, for it enables the reader to see the trends in the different fields and clearly demonstrates that there are common problems in the various phases of pest control. The antagonistic organisms that help suppress plant pathogens are similar in many respects to the natural enemies that control insect pests, and the shifting pathogens with which the plant breeder must contend present a problem very similar to insecticide resistance in insects.

The book is divided into three sections: "The Public's Stake in Pest Control," "Recent Advances in Chemical Control," and "Biological Control of Pests." In the first section there are chapters devoted to quarantine and eradication programs, regulatory control of pesticides, and some of the problems encountered in educating people how to use pesticides. This section also includes two chapters on the control of forest insects and diseases which point out the great losses that are caused by insects and diseases.

In the second section there are well written chapters dealing with recent advances in chemical control of plant diseases, weeds, and internal parasites of domestic animals. There is also a short review of the systemic insecticides of both plants and animals.

The third section which constitutes the most significant portion of the book is devoted to biological control as the term is used in its broader sense. Here for the first time are brought together such pertinent subjects as microbial control, sterile male control of the screw-worm, the use of parasites and predators in biological control, and the side effects caused by the use of pesticides. Although much has been written on these subjects, it will be helpful to have summary articles available in a single volume.

Because of the broad coverage of this book, it is understandable that all phases of pest control are not covered thoroughly or with equal emphasis. It is reasonable that a symposium be organized with emphasis on aspects of control other than chemical control of agricultural pests, for the literature on this latter subject is very extensive. However, if the purpose of this book is to show the many approaches to pest control, then surely the entomologist will find that important areas have been omitted. In the first section "The Public's Stake in Pest Control" separate chapters are devoted to the control of forest insects and forest diseases, but there is nothing included on the control of agricultural pests. Under "Recent Advances in Pest Control" the insecticides are represented by a seven-page article on systemic insecticides, but there is no discussion of the nonsystemic phosphates, the chlorinated hydrocarbon insecticides or any of the acaricides. In contrast, 30 pages are devoted to the chemical control of internal parasites of domestic animals. It also will be difficult for entomologists to consider that this book lives up to its title when four chapters are devoted to insect and disease resistance in plants and animals, and there is no mention of insect resistance to insecticides.

Entomologists and others will find this book valuable and useful if they keep in mind how difficult it would be to cover all aspects of pest control in a single volume. The editor and individual authors should be commended on the preparation of this book. The chapters have been written so they will be intelligible to readers not actively working in the special fields, and yet they will also serve as good review articles for specialists.

WILLIAM W. ALLEN
University of California
Berkeley

COMMON NAMES OF INSECTS

APPROVED BY THE ENTOMOLOGICAL SOCIETY OF AMERICA

By Jean L. Laffoon, Chairman, Committee on Common Names of Insects

This newly revised list will appear in the December 1960 issue (Vol. 6, No. 4) of the BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA.

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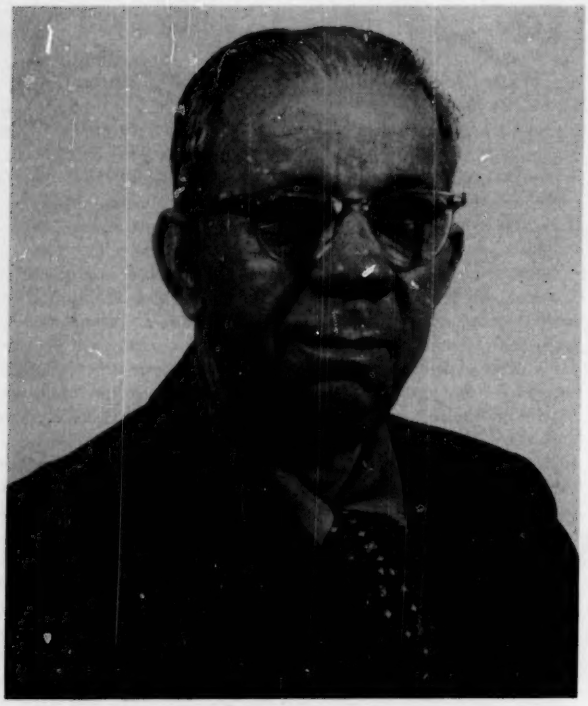


Clyde Carney Hamilton
1890–1959

Clyde Carney Hamilton
1890-1959

Dr. Clyde C. Hamilton, Research Specialist at the College of Agriculture, Rutgers University, died suddenly of a heart attack at his home in Old Bridge, New Jersey, on Sunday December 6, 1959, at the age of 69. Dr. Hamilton was born June 24, 1890 in Salina, Kansas, the son of James Edward and Anna Elizabeth (Carney) Hamilton.

He attended school in Manhattan, Kansas, and received the bachelor of science degree from Kansas State College in 1913. The University of Illinois awarded him the master of science degree in entomology in 1916. Cornell University awarded him the doctor of philosophy degree in 1930.

During World War I, Dr. Hamilton served for 8 months in the U. S. Department of Agriculture as special field agent and extension entomologist in Missouri and Arkansas. In 1918 he was appointed extension entomologist at the University of Missouri. From 1920 until 1925 he served as associate entomologist at the University of Maryland.

He was appointed to the Rutgers staff in 1925 as associate professor and associate research specialist in entomology. He has taught numerous courses in taxonomy, insect morphology and general entomology.

His research work concerning insect pests attacking ornamental plants has been published in many magazines and journals. He has written more than 50 papers on insect pests.

Dr. Hamilton was a member of the Highland Park Council from 1939 until 1943 when he resigned to serve in the War Food Administration at Washington, D. C. He returned to Rutgers in 1945.

Dr. Hamilton was a member and past president of the Eastern Branch of the American Association of Economic Entomologists. He was one of the founders of *Entoma* and served as editor during the early years when this publication was sponsored by the Eastern Branch of the Entomological Society of America. He was also a member of the National Shade Tree Conference, the American Association of Economic Entomologists, New Jersey Federation of Shade Tree Conference, the New Jersey Association of Nurserymen, and the New Jersey Greens Superintendent Association. He was a member of Sigma Xi.

The Holly Society of America recently awarded him a certificate of honor and the New Jersey Federation of Shade Tree Commissions presented him with an award of achievement.

Dr. Hamilton was a member of the Presbyterian Church of New Brunswick, and has served as a deacon, elder, and superintendent of the Sunday School.

Dr. Hamilton was married in 1918 to Vida M. Hawkins of Marysville, Kansas. He is survived by his wife and two daughters, Mrs. Jane Wooster of Columbus, Ohio, and Mrs. Carol Kreiger, Dover, Delaware.

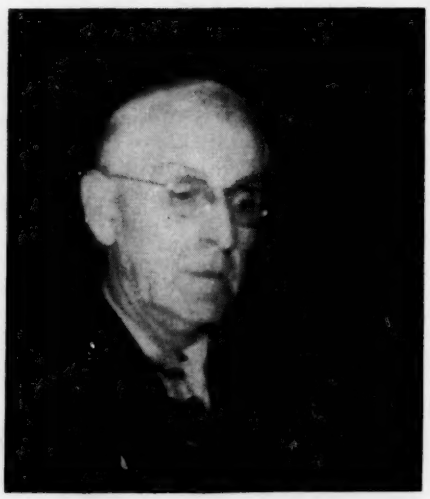
ROBERT S. FILMER

Willard Wilson Yates
1888-1960

On April 16, 1960, Willard Wilson Yates passed away suddenly at his home in Corvallis, Oregon. Mr. Yates' entire career was spent in chemical and entomological research. He leaves a host of friends and fellow workers in all parts of the country who know of his devotion and dedication to research along these lines.

Mr. Yates was born on June 16, 1888, at Juneau, Wisconsin, the son of John and Esther Yates. He attended the University of Wisconsin for 2 years before serving in the U. S. Army during World War I. An old diary discloses that he fought for 21 days in the Argonne Forest. After returning from war duty, he continued with his education and graduated from Oregon State College in 1921. From 1921 until 1926 Mr. Yates served as Assistant Chemist at Oregon State College, after which he worked for private industry for several years, returning in 1932 to receive his M.S. degree.

From 1931 to 1946 Mr. Yates was employed at Portland,



Willard Wilson Yates
1888–1960

Oregon, by the U. S. Department of Agriculture under the former Bureau of Entomology and Plant Quarantine. Most of his time was devoted to the study of mosquito problems in and around that area. He was also concerned with the encephalitis outbreak which occurred in Yakima Valley, Washington. In 1942 he was called to Orlando, Florida, for 5 months to work with other top research men of the country on the vital problem of pest control for the Army.

In 1946 his group moved from Portland to Corvallis, Oregon, which became his permanent home. He continued work on mosquito and house fly attractants, mosquito larvae sprays, and the use of radioactive isotopes in tagging insects for flight-range studies. Alone, and with other workers, he published manuscripts on these and related projects. In 1952 Mr. Yates retired from active work but always maintained a keen interest in his chosen profession.

Mr. Yates had great personal integrity; he was courteous and modest and never spoke unfavorably of anyone. His intimates recognized and appreciated his great loyalty to them and to his organization. He could be depended on to carry out research assignments no matter how much work was involved. He was an excellent team worker and took great pride in the accomplishments of the group with whom he was associated.

He was married to Phoebe Chamberlain on December 28, 1921, who survives. He is also survived by one daughter, Mrs. Melvin Shaw of Salem, Oregon; two sons, Dr. Norris Yates, a member of the faculty at Iowa State University, and Dr. Ross Yates, a member of the faculty at Lehigh University at Bethlehem, Pennsylvania; one sister, Mrs. Laura Greenfield of Portland, Oregon; and four grandchildren.

He was a member of the American Mosquito Control Association, the American Legion, the Methodist Church of Corvallis, the National Association for Retired Civil Service Employees, the Entomological Society of America, and past president of the Oregon State Entomological Society. His engrossing hobby was stamps; over a period of years he acquired an outstanding collection. He was also an active member of the Corvallis Stamp Club.

C. M. GJULLIN
A. W. LINDQUIST

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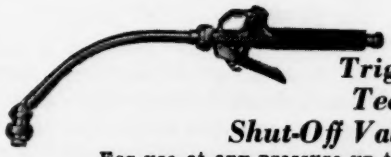

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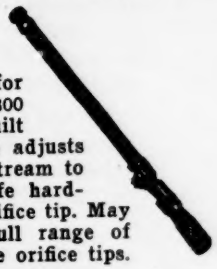

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